STUDY TITLE: β-Chloroprene: *In Vitro* Rate Constants for Metabolism in Liver, Lung, and Kidney Microsomes

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CERTIFICATION

We, the undersigned, declare that this report provides an accurate evaluation of data obtained from this study.

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TABLE OF CONTENTS

Page

CERTIFICATION **2**
TABLE OF CONTENTS **3**
LIST OF TABLES **4**
LIST OF FIGURES **4**
LIST OF APPENDICES **6**
STUDY INFORMATION **7**
SUMMARY **8**
INTRODUCTION **9**
STUDY DESIGN **9**
MATERIALS AND METHODS **9**
A. Test Substance 9
B. Test System 10
C. Microsome Preparation 10
D. Gas Chromatography Analysis 10
E. Microsomal Incubations 11
F. In Vitro Kinetic Model Description 12
G. Kinetic Parameter Point Estimation 12
H. Kinetic Parameter Probability (Bayesian) Analysis 12
RESULTS AND DISCUSSION **15**
CONCLUSIONS **16**
RECORDS AND SAMPLE STORAGE **17**
REFERENCES **17**
TABLES **19**
FIGURES **24**
APPENDICES **63**

LIST OF TABLES

Page

Table 1 Stock microsomal protein concentrations and screen for total P450 protein 20
Table 2 In vitro chloroprene metabolism prior distributions 20
Table 3 Point estimate values for the microsomal oxidation of chloroprene without correction for background loss 21
Table 4 Point estimate values for the microsomal oxidation of chloroprene with correction for background loss 22
Table 5 Probability analysis of microsomal oxidation parameters for chloroprene 23

LIST OF FIGURES

Page

Figure 1 Representative gas chromatography headspace calibration curve 26
Figure 2 Chloroprene oxidative metabolism time course in male B6C3F1 mouse liver microsomes using point estimate model parameters 27
Figure 3 Chloroprene oxidative metabolism time course in male B6C3F1 mouse lung microsomes using point estimate model parameters 28
Figure 4 Chloroprene oxidative metabolism time course in male B6C3F1 mouse kidney microsomes using point estimate model parameters 29
Figure 5 Chloroprene oxidative metabolism time course in female B6C3F1 mouse liver microsomes using point estimate model parameters 30
Figure 6 Chloroprene oxidative metabolism time course in female B6C3F1 mouse lung microsomes using point estimate model parameters 31
Figure 7 Chloroprene oxidative metabolism time course in female B6C3F1 mouse kidney microsomes using point estimate model parameters 32
Figure 8 Chloroprene oxidative metabolism time course in male Fischer rat liver microsomes using point estimate model parameters 33
Figure 9 Chloroprene oxidative metabolism time course in male Fischer rat lung microsomes using point estimate model parameters 34
Figure 10 Chloroprene oxidative metabolism time course in male Fischer rat kidney microsomes using point estimate model parameters 35
Figure 11 Chloroprene oxidative metabolism time course in female Fischer rat liver microsomes using point estimate model parameters 36
Figure 12 Chloroprene oxidative metabolism time course in female Fischer rat lung microsomes using point estimate model parameters 37
Figure 13 Chloroprene oxidative metabolism time course in female Fischer rat kidney microsomes using point estimate model parameters 38

Figure 14 Chloroprene oxidative metabolism time course in human (pooled mixed gender) liver microsomes using point estimate model parameters 39

Figure 15 Chloroprene oxidative metabolism time course in human (pooled mixed gender) lung microsomes using point estimate model parameters 40

Figure 16 Representative comparison of uniform prior and posterior distributions for human (pooled mixed gender) liver microsomal metabolism parameters 41

Figure 17 Representative comparison of uniform prior and posterior distributions for oxidative metabolism of chloroprene in human (pooled mixed gender) lung microsomes 42

Figure 18 Probability frequency of chloroprene oxidative metabolism parameters in male (M) and female (F) B6C3F1 mouse liver microsomes 43

Figure 19 Probability frequency of chloroprene oxidative metabolism parameters in male (M) and female (F) B6C3F1 mouse lung microsomes 44

Figure 20 Probability frequency of chloroprene oxidative metabolism parameters in male (M) and female (F) B6C3F1 mouse kidney microsomes 45

Figure 21 Probability frequency of chloroprene oxidative metabolism parameters in male (M) and female (F) Fischer rat liver microsomes 46

Figure 22 Probability frequency of chloroprene oxidative metabolism parameters in male (M) and female (F) Fischer rat lung microsomes 47

Figure 23 Probability frequency of chloroprene oxidative metabolism parameters in male (M) and female (F) Fischer rat kidney microsomes 48

Figure 24 Distribution of chloroprene oxidative metabolism time course in male B6C3F1 mouse liver microsomes 49

Figure 25 Distribution of chloroprene oxidative metabolism time course in female B6C3F1 mouse liver microsomes 50

Figure 26 Distribution of chloroprene oxidative metabolism time course in male Fischer rat liver microsomes 51

Figure 27 Distribution of chloroprene oxidative metabolism time course in female Fischer rat liver microsomes 52

Figure 28 Distribution of chloroprene oxidative metabolism time course in male B6C3F1 mouse lung microsomes 53

Figure 29 Distribution of chloroprene oxidative metabolism time course in female B6C3F1 mouse lung microsomes 54

Figure 30 Distribution of chloroprene oxidative metabolism time course in male Fischer rat lung microsomes 55

Figure 31 Distribution of chloroprene oxidative metabolism time course in female Fischer rat lung microsomes 56

Figure 32 Distribution of chloroprene oxidative metabolism time course in male B6C3F1 mouse kidney microsomes 57

Figure 33 Distribution of chloroprene oxidative metabolism time course in female B6C3F1 mouse kidney microsomes 58

Figure 34 Distribution of chloroprene oxidative metabolism time course in male Fischer rat kidney microsomes 59

Figure 35 Distribution of chloroprene oxidative metabolism time course in male Fischer rat kidney microsomes 60

Figure 36 Distribution of chloroprene oxidative metabolism time course in human (pooled mixed gender) liver microsomes....61

Figure 37 Distribution of chloroprene oxidative metabolism time course in human (pooled mixed gender) lung microsomes....62

LIST OF APPENDICES

Page

Appendix A Purity Analysis of β-Chloroprene Provided by the Sponsor's Supplier....64

Appendix B Human Kidney Microsome Data Sheet....67

Appendix C Gas Chromatography Gerstel Sampler Calibration Routine....69

Appendix D Gas Chromatography Gerstel Sampler Microsomal Incubation Routine....72

Appendix E Female Rodent Liver and Lung Microsomal Incubation Data Collected at DuPont Haskell Global Centers....79

Appendix F Rodent and Human Kidney Microsomal Incubation Data Collected at DuPont Haskell Global Centers....85

Appendix G Sample Model Code for MCMC Analysis....94

Appendix H Screen Capture Documentation of Model Code and Full MCMC Chain Results....105

STUDY INFORMATION

Substance Tested:
- β-Chloroprene
- 2-chloro-1,3-butadiene
- 126-99-8 (CAS Number)

Synonym(s): Chloroprene

Haskell Number: 28355

Composition: 99% β-Chloroprene (w/w)
1000 ppm p-tertiary butyl catechol (as stabilizing agent)

Purity: >99%

Physical Characteristics: Clear liquid

Stability: The test substance appeared to be stable under the conditions of the study; no evidence of instability was observed.

Study Initiated/Completed: November 14, 2007 / (see report cover page)

Experimental Start/Termination: October 9, 2007 / December 17, 2009

In-Life Initiated/Completed: April 16, 2008 / April 21, 2009

Notebook Number(s): E-111392-AF

SUMMARY

β-Chloroprene (chloroprene) *in vitro* oxidative metabolism was investigated to better understand species difference in metabolic rates among B6C3F1 mice, Fischer (F344) rats, and humans. Gas phase chloroprene concentrations were quantified by gas chromatography/micro-electron capture detection (GC/μECD). Metabolism parameters for chloroprene were estimated for mice, rats, and humans in several tissues (liver, lung, and kidney) in order to support the use of a previously published PBTK model of chloroprene for cross-species extrapolation of tumor risk based on target tissue dose. The parameters were estimated from experimental data for the metabolic clearance of chloroprene in vitro, measured in microsomal preparations. Modeling of the in vitro system was performed using a simple 2-compartment pharmacokinetic description of the in vitro system that included a non-enzymatic loss rate. Optimization of the parameter values was conducted by 2 different methods: point-estimation using the Nelder-Mead nonlinear optimization algorithm and a Bayesian statistical approach using the Markov Chain Monte Carlo algorithm. Parameter central estimates from the 2 methods were in good agreement, providing confidence in the values obtained. Estimated rates of liver metabolism based on the Bayesian approach were similar across species in terms of intrinsic clearance. The liver Vmax/Km (μmol/h/g microsomal protein) values were male mouse (195) > female mouse (145) ~ male rat (138) > human (122) > female rat (79). Lung Vmax/Km values varied substantially with male mouse (64) >> female mouse (9.7) >> male rat (1.4) ~ female rat (1.3) > human (0.3). Kidney Vmax/Km values were male mouse (17) >> male and female rat (3.3-4.2) > female mouse (0.08) > human (not detectable). These data indicate that the majority of chloroprene metabolism would be expected to occur in the liver followed the lung and kidney, with the rates in the latter 2 tissues showing notable species and sex differences. The Bayesian approach also provided estimates of the uncertainty in the parameters that can be used in the PBTK model to evaluate the uncertainty in risk estimates obtained with the model.

INTRODUCTION

Chloroprene is metabolized in mammalian systems by cytochrome P450 oxidase. The objective of this study was to develop rate constants that can be used to support physiologically based toxicokinetic modeling and identify species and sex differences relative to previously collected data for male mice and rats (Himmelstein *et al*., 2004a).[(1)] *In vitro* microsomal metabolism time course data collected at the DuPont Haskell facility were sent for kinetic modeling at the Hamner Institutes. A key component of this effort was to include parameter point estimation of the previous[(1)] and new data and apply statistical probability analysis to define parameter variation.

STUDY DESIGN

The table below outlines the key tasks performed for this study.

Task	Species	Sex	Tissue	Endpoints
Prepare microsomes and measure metabolism	Mouse	Male	kidney	Protein concentration, Total P450, Chloroprene concentration time course by GC/μECD
		Female	liver, lung, kidney	
	Rat	Male	kidney	
		Female	liver, lung, kidney	
	Human	Pooled	kidney	
Describe in vitro model	(start with Himmelstein *et al*., 2004)[(1)]			Documentation of model code
Conduct parameter point estimation	(by ACSL Optimize)[a]			Vmax, Km & Vmax/Km[b]
Conduct probability analysis	(by Markov Chain Monte Carlo analysis)			Vmax, Km & Vmax/Km[c]

a Included re-analysis of B6C3F1 mouse, F344 rat, and human chloroprene microsomal oxidation data for male liver and lung microsomes from Himmelstein *et al*. (2004a).
b As point estimates
c As geometric mean (GM) and standard deviation (GSD)

MATERIALS AND METHODS

A. Test Substance

The test substance, β-Chloroprene (chloroprene), was supplied as a clear liquid by DuPont Performance Elastomers, Pontchartrain Works (LaPlace, Lousiana, U.S.A.). A representative purity analysis provide by the sponsor is shown in Appendix A. It contained p-tertiary butyl catechol as a stabilizing agent which subsequently was removed by filtration through activated alumina under nitrogen atmosphere as described previously (Himmelstein *et al*., 2001).[(2)] The purified chloroprene was stored at < -70°C under nitrogen headspace atmosphere. The test substance appeared to be stable under the conditions of the study. No evidence of instability, such as a change in color or physical state, was observed.

For metabolism experiments, vapor concentrations were prepared by adding the liquid test substance to Tedlar® bags (SKC Inc., Eighty Four, Pennsylvania, U.S.A.) containing a known volume of room air (MW 88.5365 g/mol and 0.9598 g/cm³ liquid, 3.8 µL/L air for 1000 ppm). Further gas phase dilutions were made for calibration or exposure purposes. Gas tight syringes were used for the gas transfers.

B. Test System

Fischer rat (F344/DuCrl) and mice (B6C3F1/Crl) were received from Charles River Laboratories, Inc., Raleigh, North Carolina. The species and strains were selected to match those used for inhalation toxicity testing by the National Toxicology Program (NTP 1998).[3] The animals were maintained in solid bottom cages with rodent chow (Certified Rodent LabDiet® 5002, PMI® Nutrition International, LLC, St. Louis, Missouri, U.S.A.) and water *ad libitum,* and acclimated for at least 7 days prior to use. Laboratory facilities were fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). All procedures involving animals were reviewed by the laboratory animal care and use committee. A total of 15 female rats and 50 female mice were used for preparation of the liver and lung microsomes. A total of 15 rats/sex and 30 female mice/sex were used for preparation of kidney microsomes.

Human kidney microsomes were purchased from Xenotech (Lenexa, Kansas, U.S.A.). The vendor supplied data sheet is given in Appendix B.

C. Microsome Preparation

Female mice received on October 9, 2007 were 12.7 weeks of age at the time the liver and lung microsomes were prepared. Female rats received on November 1, 2007 were 10.9 weeks when liver and lung microsomes were made. For kidney microsomes, the male and female mice and rats were received on January 29, 2009 and were 11.9 weeks of age when the microsomes were made. Lung and liver microsomes were prepared by differential centrifugation as described by Himmelstein *et al*. (2004a).[1] The microsomal preparations were analyzed for protein by the Bradford (1976)[4] method (Bio-Rad Laboratories, Hercules, California, U.S.A.). The P450 content was measured by spectrophotometry using established methods (Omura and Sato, 1964; Guengerich, 1982).[5,6] All fractions were stored at <-70°C. Stock protein (mg protein/mL) and total P450 (nmol/mg protein) are summarized in Table 1.

D. Gas Chromatography Analysis

Gas chromatography (GC) was used for quantitative analysis of chloroprene. The GC method parameters are summarized in the table below. The method was similar to the one used previously except that micro-electron capture detection (µECD) was used in place of mass spectrometry single ion monitoring (Himmelstein *et al*., 2004).[1] The µECD was used because it gave adequate sensitivity for quantitation of the parent chemical. Data on the concentration of the epoxide metabolite was not collected in the current experiments because of the focus on total chloroprene metabolism as a dosimetric for dose-response modeling (Himmelstein *et al*. 2004b).[7]

Samples were injected on the GC using a robotic x-y-z programmable multipurpose sampler (MPS2, Gerstel US, Baltimore, Maryland, U.S.A.) with a headspace injection volume of 200 µL. Calibrations were performed over a range of concentrations (0.25 – 604 ppm) and based on peak area responses for a 5-point calibration. A standard curve was generated and checked on each day of analysis.

Instrument:	Agilent HP6890 equipped with Gerstel MP2 autosampler
Gerstel:	
Syringe:	1 mL headspace, heated
Calibration conditions:	See Appendix C
Incubation conditions:	See Appendix D
Inlet:	
Type:	Split
Temperature:	175°C
Pressure:	4.05 psi
Split ratio:	5:1
Split flow:	21.2 mL/min
Total flow:	28.4 mL/min
Gas type:	Helium
Column:	
Type:	JW 125-5032, 30 m x 530 µm, 1.5 µm film thickness
Mode:	Constant pressure
Pressure:	4.05 psi
Nominal initial flow:	4.2 mL/min
Average velocity:	35 cm/sec
Method:	Isothermal at 100°C
Detector:	
Type:	µECD
Temperature:	250°C
Mode:	Constant makeup flow
Make-up flow:	30 mL/min
Make-up gas type:	Argon (95%):methane (5%)
Retention time:	Approximately 2.1 minutes

E. Microsomal Incubations

The total volume of Gerstel 10-mL vials used for the incubations was confirmed by gravimetric displacement with water. The measuresment was made on 2 occasions once for the liver and lung microsome incubations (n=10 vials) and once for the kidney microsome incubations (n=10 vials). The respective mean (±SD) weights when filled completely with water at room temperature were 11.648 (±0.222) and 11.634 (±0.051) grams. These values were used directly (without correction for the specific gravity of water) to calculate the corresponding headspace volumes less the 1.0 mL used for the incubation liquid phase.

The time course of chloroprene disappearance was measured in liver, lung and kidney microsomes using the method described in Himmelstein *et al*. (2004a).[(1)] Vials were prepared

with 0.1 M phosphate buffer (pH 7.4), $MgCl_2$ (15 mM), EDTA (0.1 mM), glucose-6-phosphate (10 mM), and glucose-6-phosphate dehydrogenase (2 U/mL). Incubations were started by the addition $NADP^+$ (0.53 mM). Control incubations were run in the absence of $NADP^+$ by addition of an equal volume of phosphate buffer. Representative incubation conditions are given for female mouse liver (Appendix E) and for male mouse kidney (Appendix F). Microsomal protein concentrations were established from previous work (liver and lung) or experimentally for kidney microsomes (Appendix F). Definitive experiments used protein concentrations that ranged from 1-3 mg/mL. The injection volume was established during methods development. Headspace samples (200 µL) were injected via auto-sampler at 0, 12, 24, 36, 48, and 60 minutes. Area counts were recorded and headspace concentrations (nmol/mL) were calculated in Microsoft® Office Excel 2003.

F. In Vitro Kinetic Model Description

A 2-compartment model modified from Himmelstein *et al*. (2004a)[1] was used to describe the time-concentration measurements of chloroprene in the headspace in the closed vial system. The current model describes the loss of chloroprene from the headspace as 1) background loss rate and 2) microsomal oxidation only (1-CEO hydrolysis pathway was turned off). To estimate the gender-specific variability of the kinetic parameters, male tissue data from Himmelstein *et al*. (2004a)[1] were re-evaluated in the parameter optimization process. For a more detailed description of the male dataset and the 2-compartment model, see Himmelstein *et al*. (2004a).[1]

G. Kinetic Parameter Point Estimation

To quantitatively compare the more commonly used point-estimation technique with the Bayesian approach, all model parameters were optimized with ACSL-Optimize (version 11.8.4, AEgis, Technologies Group, Inc, Huntsville, Alabama, USA), using the Nelder-Mead method with a relative error minimization-based, log-likelihood function.

H. Kinetic Parameter Probability (Bayesian) Analysis

In addition to the traditional point estimation, Bayesian analysis was performed to evaluate the uncertainty and variability of the metabolic parameters. Bayesian analysis is a statistical procedure which estimates the model parameters of an underlying distribution based on the likelihood of the previous knowledge (prior distributions) and observed data. When gender-specific tissue data were available (i.e., rat and mouse), a 2-level hierarchical Bayesian model was used to estimate the gender-variability of the metabolic parameters.[a]

This approach was hierarchical in the sense that the uncertain population level (species) parameters at the top level define the variability of the lower-level (gender) parameter values, which in turn predict the headspace concentrations in each experiment as a function of the initial

[a] Bayesian analysis can be implemented using a Markov chain Monte Carlo (MCMC) algorithm which updates the prior distributions based on the posterior likelihoods. For each iteration, the model proposes new values for the parameters (one at a time), re-computing the posterior likelihood. When the posterior likelihood for the new proposed value is relatively good compared to the likelihood for the current parameter values, it is more likely to be accepted. In this way, the probability of moving from one set of proposed values to another depends on how "good" the proposed values are. Better parameter values tend to be accepted more often than inferior parameter values and thus an approximation of the posterior density for the parameters is obtained.

chloroprene exposure concentraiton. For incubation of chloroprene with human tissue microsomes, only population-level parameters were estimated since there was only mixed gender data available. An example of the model code for one of the MCMC analyses is provided in Appendix G.

1. Definition of Prior Distributions

Inter-gender variability for a given microsomal activity parameter (in log-scale) was described by a normal distribution with the population mean M and the standard deviation S. The prior distribution of M was modeled by drawing M from a uniform distribution (Table 2). The same log-uniform distributions were used for Vmax, Km and Vmax/Km for all the animal species, tissues, and doses. It was assumed that the log-uniform distribution [-10, 5], with lower bound 4.5e-5 and upper bound 148, was broad enough to encompass the actual distributions of the metabolic parameters. The values were determined from the point estimation results in Himmelstein *et al*. (2004a)[(1)], and 2 preliminary MCMC analyses. Before a fixed log-uniform distribution [-10, 5] was selected, 2 uniform distributions were tested for microsomal activity parameters; one [1e-8, 500] (natural scale); and the other [-20, 10] (log-scale). All 3 "prior" types produced the identical posterior results given the same variability and error model. The log-uniform [-10, 5] was chosen to reduce the sampling time.

Prior descriptions of the gender-specific variability (S) were chosen to be lognormal [0.3, 5]. Because the MCMC parameters were sampled in log-space, the estimated gender-specific variability was an equivalent description to the coefficient of variation. One additional distribution, lognormal [0.3, 1], was tested in the preliminary analysis. Given the same prior conditions on other parameters, the posterior results obtained from the 2 priors for gender-specific variability were very compatible. The broader prior (lognormal [0.3, 5]) was selected to avoid over-constraining the parameters. For each gender, the individual-level parameter (m) was sampled from the population distribution (Norm (M,S)). The exponential form of the individual parameters (exp(m)) were then used as the inputs to compute the 2-compartment PK model predictions. The data likelihoods (likelihoods of getting the observed data given the individual parameter values) were calculated by assuming that the log-transformed predictions were normally distributed around the log-transformed M with a variance of δ^2. The prior distribution for δ was defined as Normal [1,1].

2. MCMC Computation Process

MCMC process is a computationally intensive search for parameter values and updating prior distributions based on posterior likelihoods. The following steps were performed in the each MCMC iteration leading to probability based estimates of Vmax, Km or Vmax/Km:

Step	Computation
A	Sample population parameter 'M' from the prior distribution
B	Sample gender-specific variability 'S' from the prior distribution
C	Sample gender-specific parameter 'm' from Norm (M, S)
D	Calculate metabolic parameter (Vmax, Km or Vmax/Km) as exp(m)
E	Compute the model predictions with the updated model parameters
F	Compute the posterior likelihood with each new updated parameter based on their prior distributions, and the experimental data
G	Repeat steps d-f for each gender
H	Repeat steps a-g for each MCMC iteration until convergence of the posterior distributions of M and m is reached.

The method of Brooks and Gelman (1998)[8] was used to diagnose the convergence of MCMC chains. Presentation of the process included probability frequencies, mean (exp(m)) and standard deviation (std(exp(m))) estimates of the 50th percentile central tendencies, time course plots of chloroprene headspace concentrations with model estimates as a distribution of 50 simulation samples. In addition to the example MCMC model code given in Appendix G, a summary of the full MCMC file collection is presented in Appendix H.

RESULTS AND DISCUSSION

Stock protein concentrations were highest for the liver microsomes (ranging from 25.6 to 34.6 mg/mL) with lower concentrations in the lung (6.1-8.4 mg/mL) and kidney (6.0-10.0 mg/mL) (Table 1). The P450 content likewise was highest in the liver, followed by the kidney, and non-detected levels in the lung. The non-detectable P450 content was most likely because of the lack of sensitivity of the carbon monoxide binding (spectrophotometric) assay (LOD ~ 0.02 nmol/mg protein). The stock proteins were diluted to 1-3 mg/mL of total incubation volume for studying the rate of chloroprene metabolism. Metabolic uptake (disappearance from the headspace) was initially characterized qualitatively as the percent change between the starting and final measured concentration (Appendices E-F). Human kidney microsomes were the only tissue that showed no discernable decline over the 60 minute incubation period (Appendix F). The metabolism of chloroprene could be easily detected in liver, and although at a slower rate in lung and kidney incubations as well. The concentration time course data, except for the human kidney, were determined adequate for in vitro modeling based on comparison of percent uptake relative to the control incubations (no $NADP^+$).

A Bayesian statistical approach using the Markov Chain Monte Carlo (MCMC) algorithm was adapted to analyze in vitro chloroprene metabolism data. Nine MCMC analyses were performed for this study (control dataset for background loss rate; liver, lung, and kidney for rat and mouse, and liver and lung for human), using the "prior" distributions summarized in Table 2. The human kidney microsomal metabolism data was not modeled because of the failure to produce experimentally measurable chloroprene uptake. Three MCMC chains were run for each analysis. A minimum of 200,000 iterations were performed for each chain. The first 100,000 iterations were used to initialize the Monte Carlo chain, and these results were used as the starting point for completion of the remaining 100,000 iterations. Once the MCMC chains converged to a stationary distribution, the "converged" parts of the chains were considered representative samples from the posterior distributions. The MCMC chains were considered converged when the estimates of the corrected scale reduction factor (CSRF) were close to 1; a value of 1.2 was selected as a cut-point for determining convergence. The CSRF values (abbreviated as R in the model) for all the parameters were below 1.1. After the chains converged, 4000 sets of the parameters were randomly sampled from the converged part of the chains to represent the posterior distributions. Intrinsic clearance (Vmax/Km) was calculated from the geometric mean values for Vmax and Km.

The posterior distribution for the background loss rate (in addition to removal of chloroprene during headspace sample extraction) was based on 8 sets of control data (the complete female data set plus the male kidney dataset). A prerequisite assumption was that the in vitro experimental background loss rate was independent of gender, tissue, and dose. The first-order rate constant included in the model to account for the background loss was based on the resulting posterior distribution [95^{th}, 50^{th} and 5^{th} percentile of 1.5, 1.4, and 1.3 L/hr/g, respectively].

For comparison with the Bayesian analysis, the traditional Nelder-Mead optimization routine for model parameter optimization was run using ACSL-Optimize. The point estimate of the background loss rate was 1.41 L/hr/g using ACSL-Optimize. The point estimation results for microsomal oxidation parameters with and without background loss rate are presented in

Tables 3 and 4, respectively. Even with the background loss rate, microsomal oxidation was still predicted to occur in most of the tissues. In some of the low metabolism tissues it was possible to see an impact of considering the background loss; for example the estimated intrinsic clearance dropped from 1.3 to 0.9 L/hr/g in the male rat lung microsomal incubations. The greatest impact was for the female mouse kidney where the intrinsic clearance dropped from 0.83 to 0.024 L/hr/g, which was essentially negligible. Figures 2-15 show the comparison of chloroprene headspace measurements and predictions simulated using point estimates of the model parameters (Table 4).

Posterior distributions of the model parameters showed excellent agreement with the point estimates (Table 4). The point estimates were typically within one standard deviation of the MCMC mean values (Table 5), providing a cross-validation between the 2 optimization techniques. One exception was for Vmax/Km in the human lung, where the Bayesian estimate (0.3) was somewhat lower that the point estimate (0.9). The uncertainties in the model parameters were significantly reduced from the prior distributions (Figures 16 and 17) demonstrated by the narrow posterior distribution. For all species, microsomal activities were highest in the liver, followed by lung or kidney (Tables 4 and 5). Gender differences in the estimated parameter values were observed in all tissues for which the necessary data were available (Table 4); for example, the intrinsic clearance (Vmax/Km) for liver microsomes was higher in male than female animals for both rats and mice. Figures 18-23 compare the posterior distributions of metabolic parameters in male and female. Species differences on the tissue intrinsic clearance rates were also observed. Higher clearance was estimated in microsomal incubation with the lung than kidney for mice; but this was reversed for rats (Table 4 and 5). Figures 24-37 present the distributions of chloroprene predictions simulated using model parameters randomly drawn from their posterior distributions. The width of the band showing 50 randomly selected simulations reflects the impact of the uncertainty and variability of the metabolic parameters on the distribution of the model output (chloroprene concentration). As a result the point estimate and Bayesian approaches gave simulations that well represent the experimental in vitro concentration time course data (Figures 2-15 and 24-37) with the latter approach providing estimates of parameter uncertainty.

CONCLUSIONS

Metabolism parameters for chloroprene were estimated for mice, rats, and humans in several tissues (liver, lung, and kidney) in order to support the use of a previously published PBTK model of chloroprene for cross-species extrapolation of tumor risk based on target tissue dose. The parameters were estimated from experimental data for the metabolic clearance of chloroprene in vitro, measured in microsomal preparations. Modeling of the in vitro system was performed using a simple 2-compartment pharmacokinetic description of the in vitro system that included a non-enzymatic loss rate. Optimization of the parameter values was conducted by 2 different methods: point-estimation using the Nelder-Mead nonlinear optimization algorithm and a Bayesian statistical approach using the Markov Chain Monte Carlo algorithm. Parameter central estimates from the 2 methods were in good agreement, providing confidence in the values obtained. Estimated rates of liver metabolism based on the Bayesian approach were similar across species in terms of intrinsic clearance. The liver Vmax/Km (μmol/h/g microsomal protein) values were male mouse (195) > female mouse (145) ~ male rat (138) > human (122) >

female rat (79). Lung Vmax/Km values varied substantially with male mouse (64) >> female mouse (9.7) >> male rat (1.4) ~ female rat (1.3) > human (0.3). Kidney Vmax/Km values were male mouse (17) >> male and female rat (3.3-4.2) > female mouse (0.08) > human (not detectable). These data indicate that the majority of chloroprene metabolism would be expected to occur in the liver followed the lung and kidney, with the rates in the latter 2 tissues showing notable species and sex differences. The Bayesian approach also provided estimates of the uncertainty in the parameters that can be used in the PBTK model to evaluate the uncertainty in risk estimates obtained with the model.

RECORDS AND SAMPLE STORAGE

Specimens (if applicable), raw data, the protocol, amendments (if any), and the final report will be retained at DuPont Haskell, Newark, Delaware, Iron Mountain Records Management, Wilmington, Delaware, or Quality Associates Incorporated, Fulton, Maryland.

Laboratory-specific raw data such as personnel files, instrument, equipment, refrigerator and/or freezer raw data will be retained at the facility where the work was done.

REFERENCES

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TABLES

Table 1
Stock microsomal protein concentrations and screen for total P450 protein

Species	Sex	Tissue	Stock protein (mg/mL)	P450 (nmol/ mg protein)
B6C3F1 mouse	Male	Kidney	6.945	0.197
	Female	Liver	34.648	0.445
		Lung	8.429	ND
		Kidney	5.965	ND
F344 rat	Male	Kidney	9.826	0.022, 0.128[a]
	Female	Liver	25.555	0.519
		Lung	6.118	ND
		Kidney	9.514	0.048
Human	Mixed	Kidney	10.0[b]	ND

ND - not detected

a Measurement taken on separate days indicated variation in spectral analysis.
b Concentration provided by Xenotech

Table 2
In vitro chloroprene metabolism prior distributions

Parameter application	Vmax, Km, Vmax/Km[a]	
	Distribution	Truncation
Population (exp(M))	Uniform	[4.5e-5, 150]
Gender variability (S)	Lognormal (0.3, 5)	[0.01, 10]
Individual[b] (exp(m))	Exp(Normal (M, S))	[2e-9, 2e4]

M - Mean; exp(M) - exponent of mean, S - standard deviation

a Units: Vmax (µmol/h/mg), Km (µmol/L), Vmax/Km (L/hr/g protein)
b Individual level parameter referred to male-specific and female-specific metabolic parameter in the 2-compartment PK model

Table 3
Point estimate values for the microsomal oxidation of chloroprene without correction for background loss

			Activity of microsomal oxidation[a,b]		
Species	Sex	Tissue	Vmax	Km	Vmax/Km
B6C3F1 mouse	Male	Liver	0.23	1.03	224
		Lung	0.10	1.5	66.7
		Kidney	0.0137	0.73	18.8
	Female	Liver	0.12	0.9	133
		Lung	0.03	2.81	11
		Kidney	0.0015	1.81	0.83
F344 rat	Male	Liver	0.078	0.53	146
		Lung			1.3
		Kidney	0.0057	1.87	3.05
	Female	Liver	0.062	0.57	109
		Lung			2.6
		Kidney	0.0022	0.37	5.95
Human	Mixed	Liver	0.1	1.5	101
		Lung			1.3

a Obtained by ACSL Optimization
b Vmax (µmol/h/mg); Km (µmol/L); Vmax/Km (L/hr/g)

Table 4
Point estimate values for the microsomal oxidation of chloroprene with correction for background loss

			Activity of microsomal oxidation[a,b]		
Species	Sex	Tissue	Vmax	Km	Vmax/Km
B6C3F1 mouse	Male	Liver	0.26	1.36	186
		Lung	0.13	2.0	64
		Kidney	0.01	0.5	20
	Female	Liver	0.09	0.53	174
		Lung	0.025	2.78	8.9
		Kidney	0.00004	1.7	0.024
F344 rat	Male	Liver	0.077	0.56	139
		Lung			0.9
		Kidney	0.0027	0.92	3
	Female	Liver	0.068	0.82	82
		Lung			1.2
		Kidney	0.00177	0.37	4.7
Human	Mixed	Liver	0.054	0.45	120
		Lung			0.9

a Obtained by ACSL Optimization and includes correction for background loss of chloroprene during the incubation
b Vmax (µmol/h/mg); Km (µmol/L); Vmax/Km (L/hr/g)

Table 5
Probability analysis of microsomal oxidation parameters for chloroprene

			Activity of microsomal oxidation[a]					
			Vmax[b]		Km[b]		Vmax/Km[c]	
Species	Sex	Tissue	Mean	SD	Mean	SD	Mean	SD[d]
B6C3F1 mouse	Male	Liver	0.27	0.010	1.37	0.08	194.7	14.2
		Lung	0.14	0.007	2.23	0.14	63.7	5.0
		Kidney	0.013	0.001	0.77	0.08	16.8	2.3
	Female	Liver	0.123	0.010	0.85	0.12	144.5	23.0
		Lung	0.026	0.010	2.68	1.29	9.7	6.0
		Kidney	0.0003	0.0013	3.74	20.8	0.08	0.57
F344 rat	Male	Liver	0.077	0.002	0.56	0.03	138.0	7.9
		Lung					1.4[e]	0.2
		Kidney	0.0025	0.0003	0.77	0.12	3.3	0.6
	Female	Liver	0.076	0.004	0.97	0.06	78.6	6.8
		Lung					1.3[e]	0.2
		Kidney	0.0025	0.0003	0.60	0.08	4.2	0.7
Human	Mixed	Liver	0.055	0.001	0.45	0.02	122.2	4.8
		Lung					0.33[e]	0.22
		Kidney					ND[f]	

a Mean (exp(m)) and standard deviation SD (exp(s)) values obtained by Markov Chain Monte Carlo (MCMC) analysis and includes correction for background loss of chloroprene during the incubation
b Vmax (µmol/h/mg); Km (µmol/L)
c Vmax/Km (L/hr/g) calculated as Vmax/Km*1000 mg/g (unit conversion)
d Except as noted, SD = Vmax/Km * Squareroot((Vmax SD/Mean)²+(Km SD/Mean)²) (Taylor, J.R. (1982). An Introduction to Error Analysis: The Study of Uncertainties in Physical Measurements. University Science Books, Mill Valley.)
e Mean and SD Vmax/Km estimated directly via MCMC analysis
f ND – metabolism not detected

FIGURES

FIGURES

EXPLANATORY NOTES

ABBREVIATIONS:

Frequenc - frequency

Figure 1
Representative gas chromatography headspace calibration curve

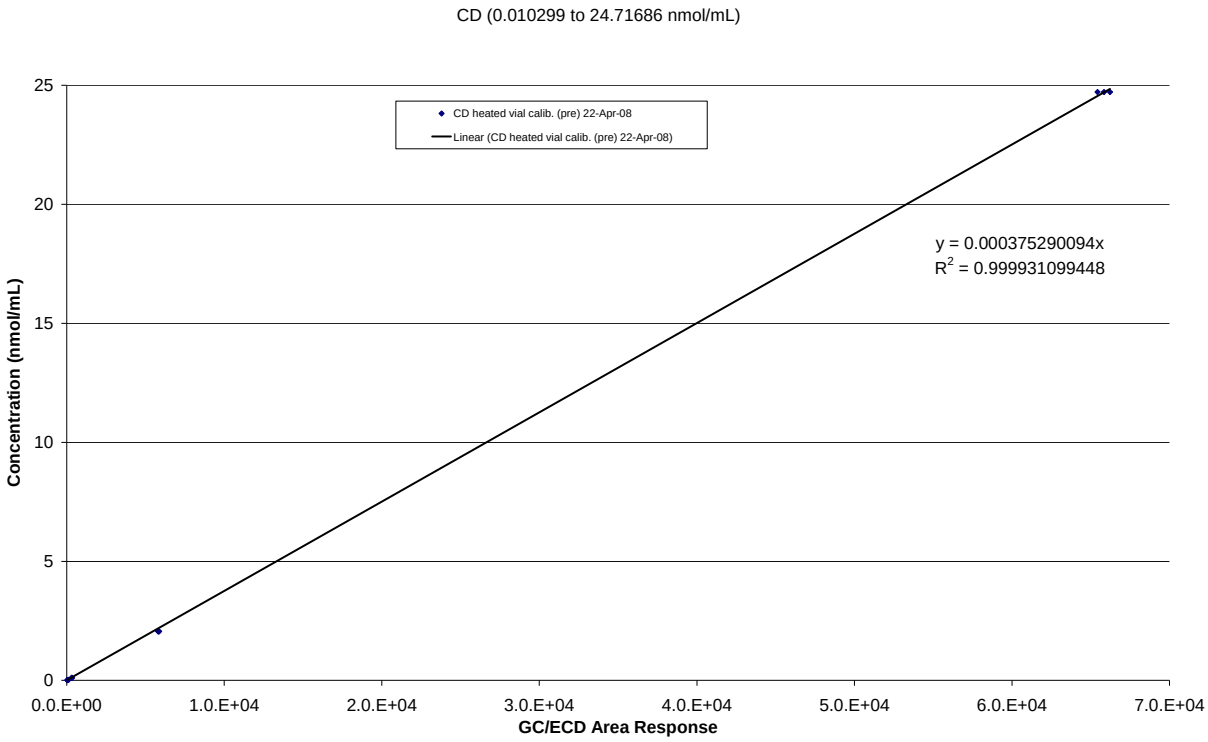
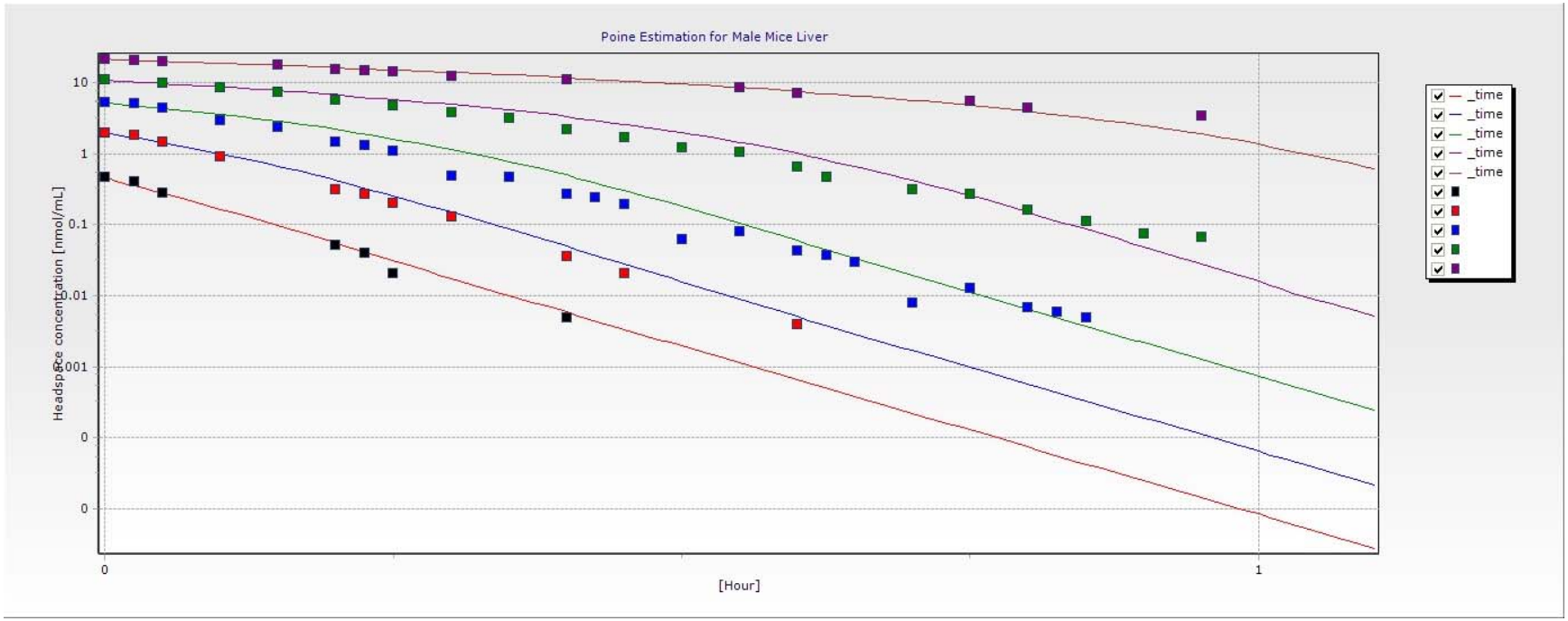
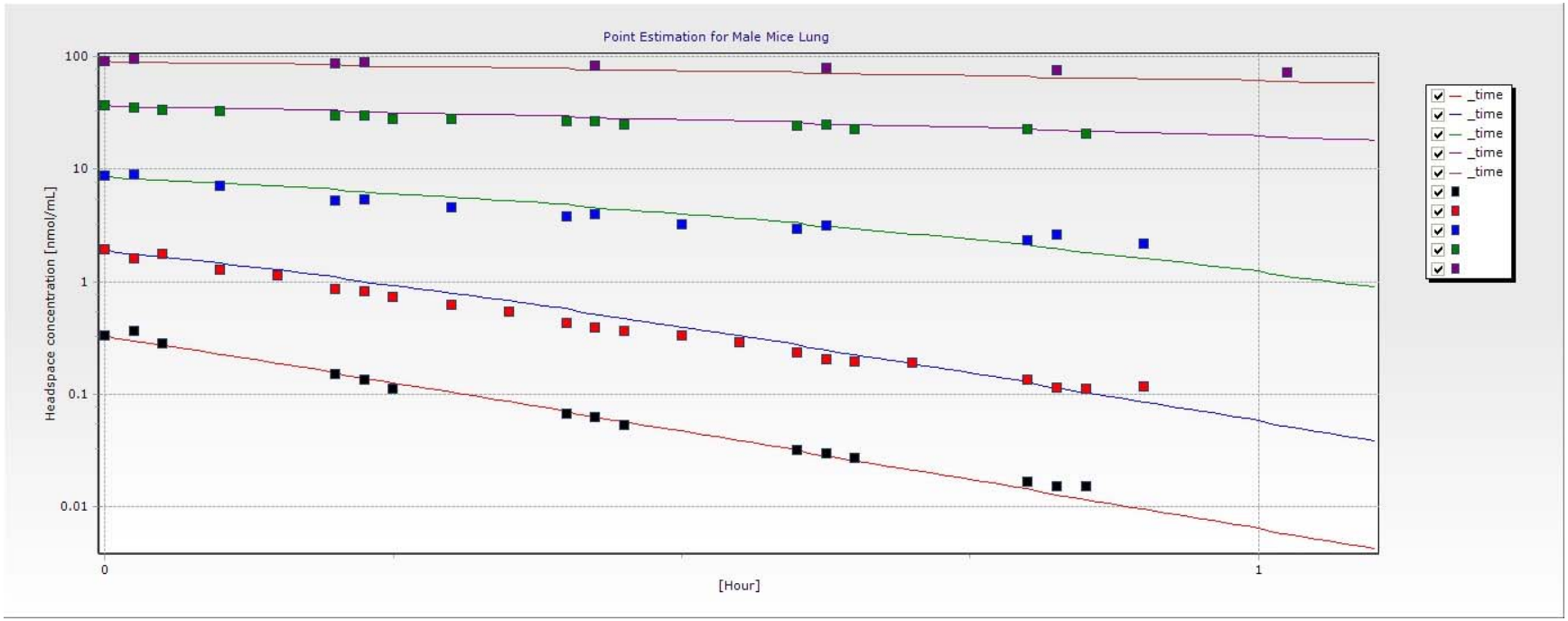


Figure 2
Chloroprene oxidative metabolism time course in male B6C3F1 mouse liver microsomes using point estimate model parameters



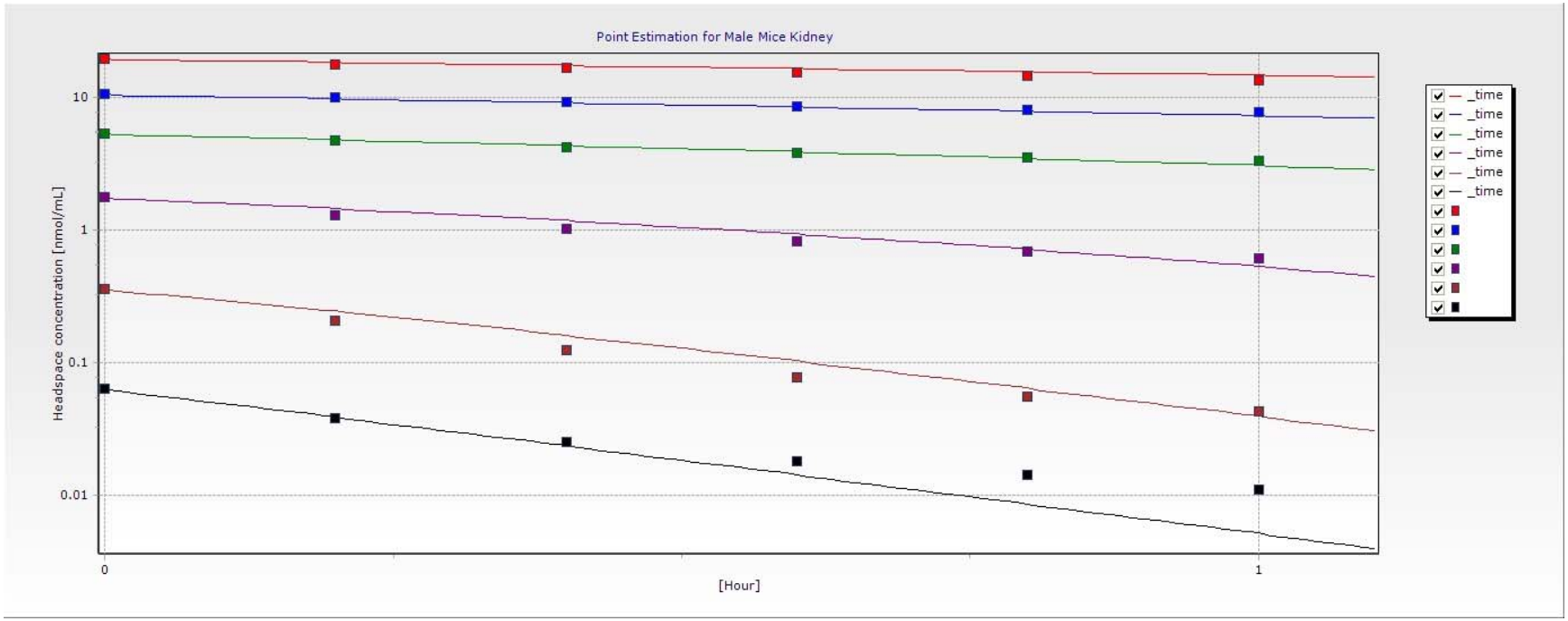
Note: Chloroprene headspace concentrations (symbols) and model simulations (lines) based on parameter values reported in Table 4.

Figure 3
Chloroprene oxidative metabolism time course in male B6C3F1 mouse lung microsomes using point estimate model parameters



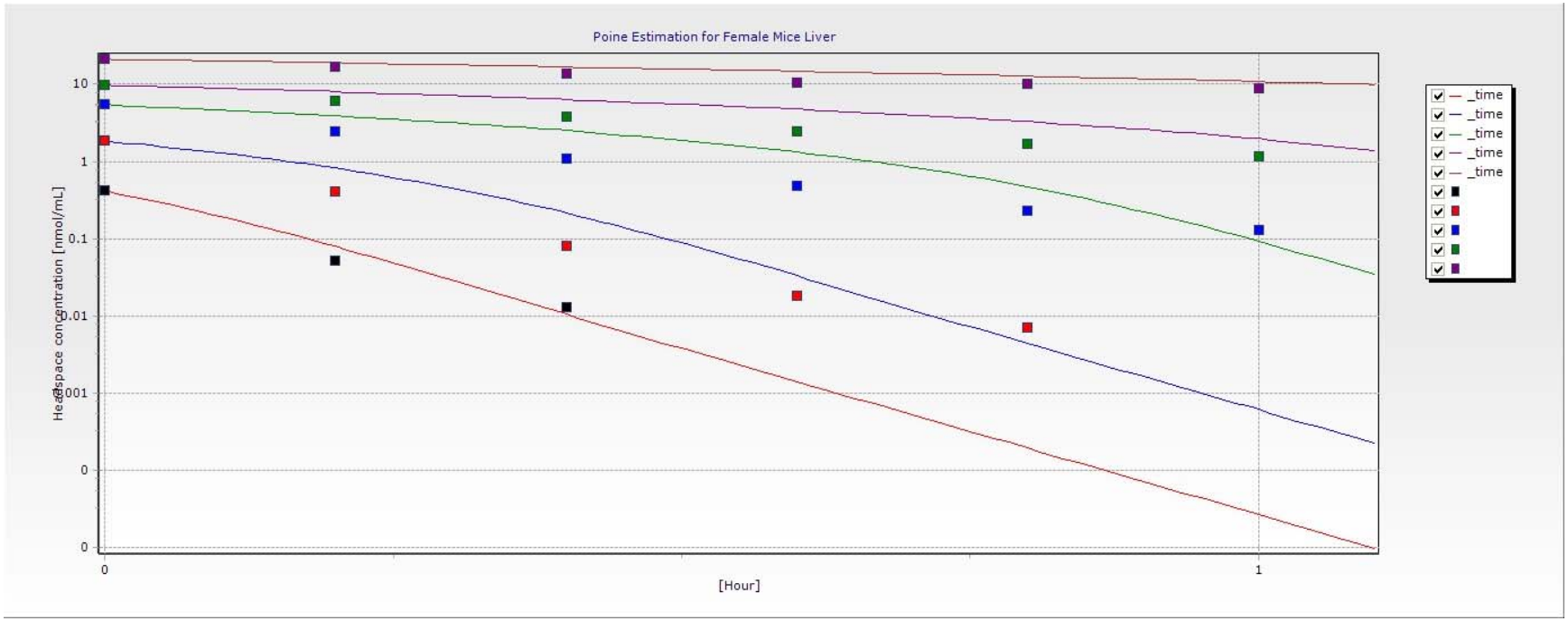
Note: Chloroprene headspace concentrations (symbols) and model simulations (lines) based on parameter values reported in Table 4.

Figure 4
Chloroprene oxidative metabolism time course in male B6C3F1 mouse kidney microsomes using point estimate model parameters



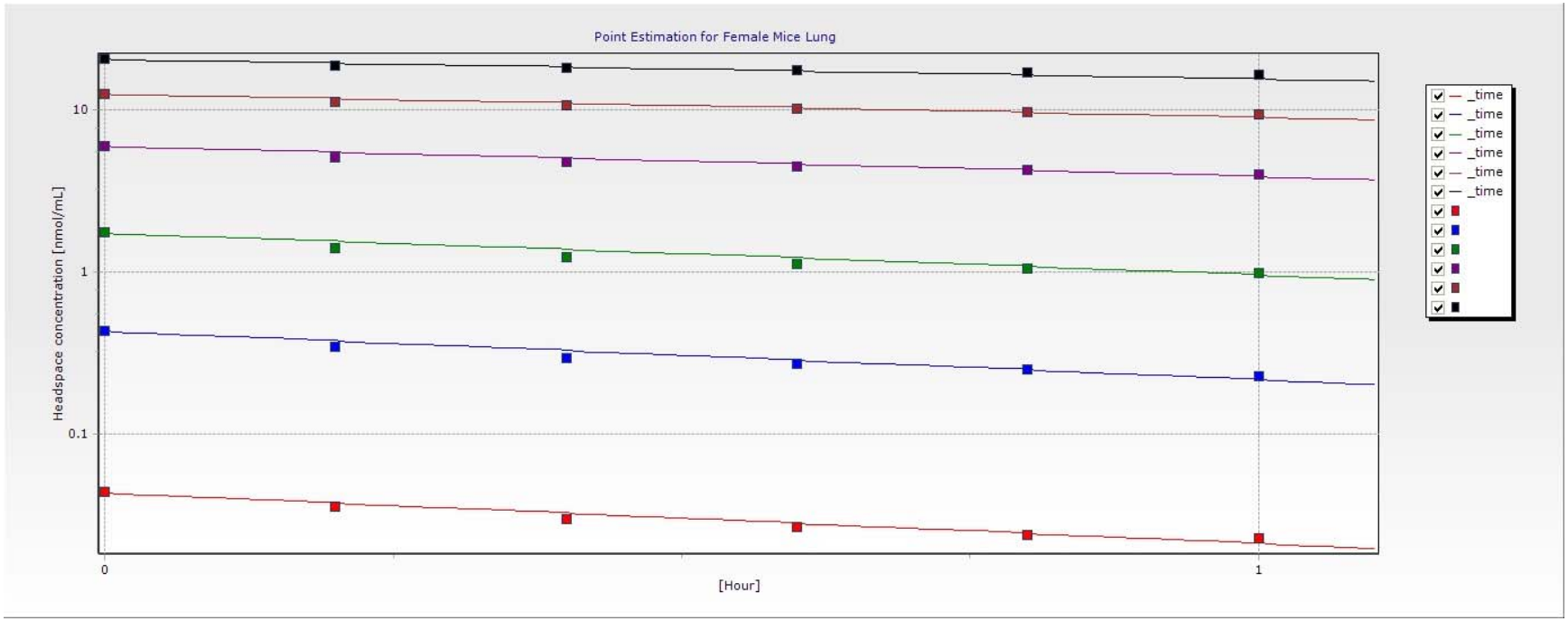
Note: Chloroprene headspace concentrations (symbols) and model simulations (lines) based on parameter values reported in Table 4.

Figure 5
Chloroprene oxidative metabolism time course in female B6C3F1 mouse liver microsomes using point estimate model parameters



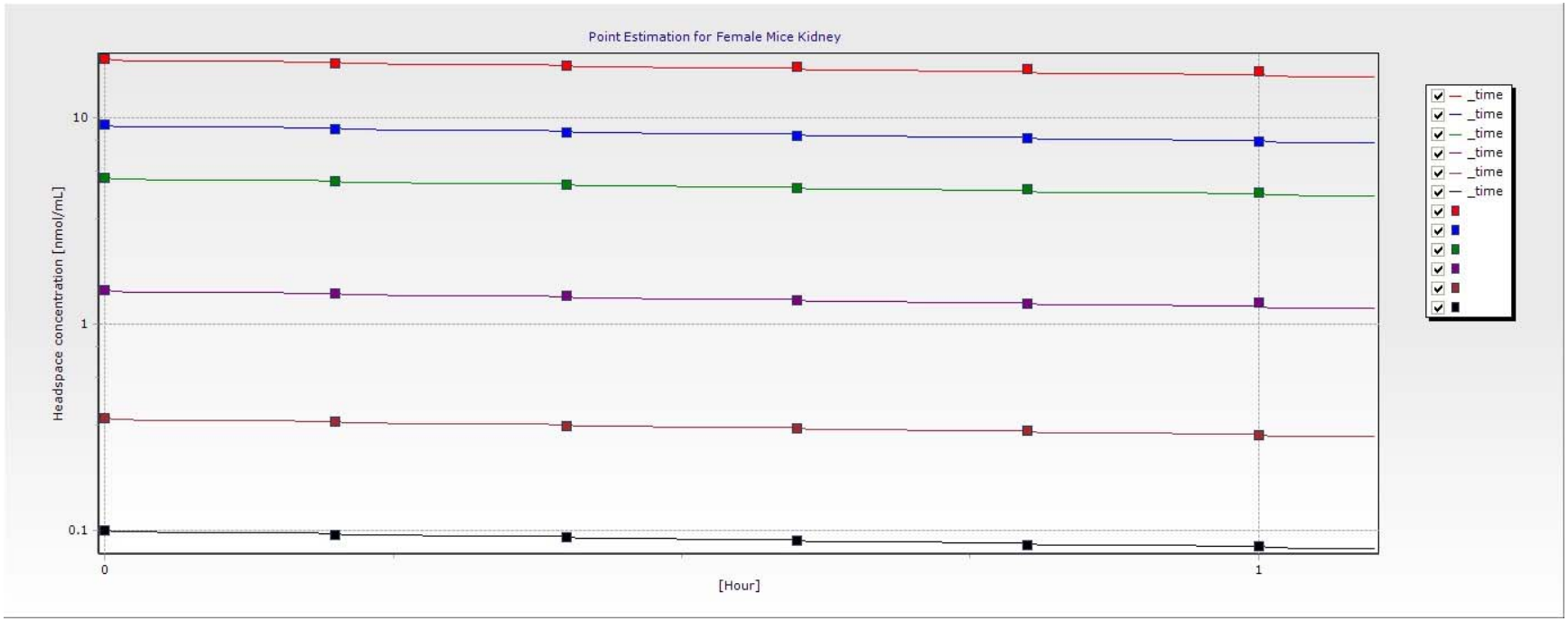
Note: Chloroprene headspace concentrations (symbols) and model simulations (lines) based on parameter values reported in Table 4.

Figure 6
Chloroprene oxidative metabolism time course in female B6C3F1 mouse lung microsomes using point estimate model parameters



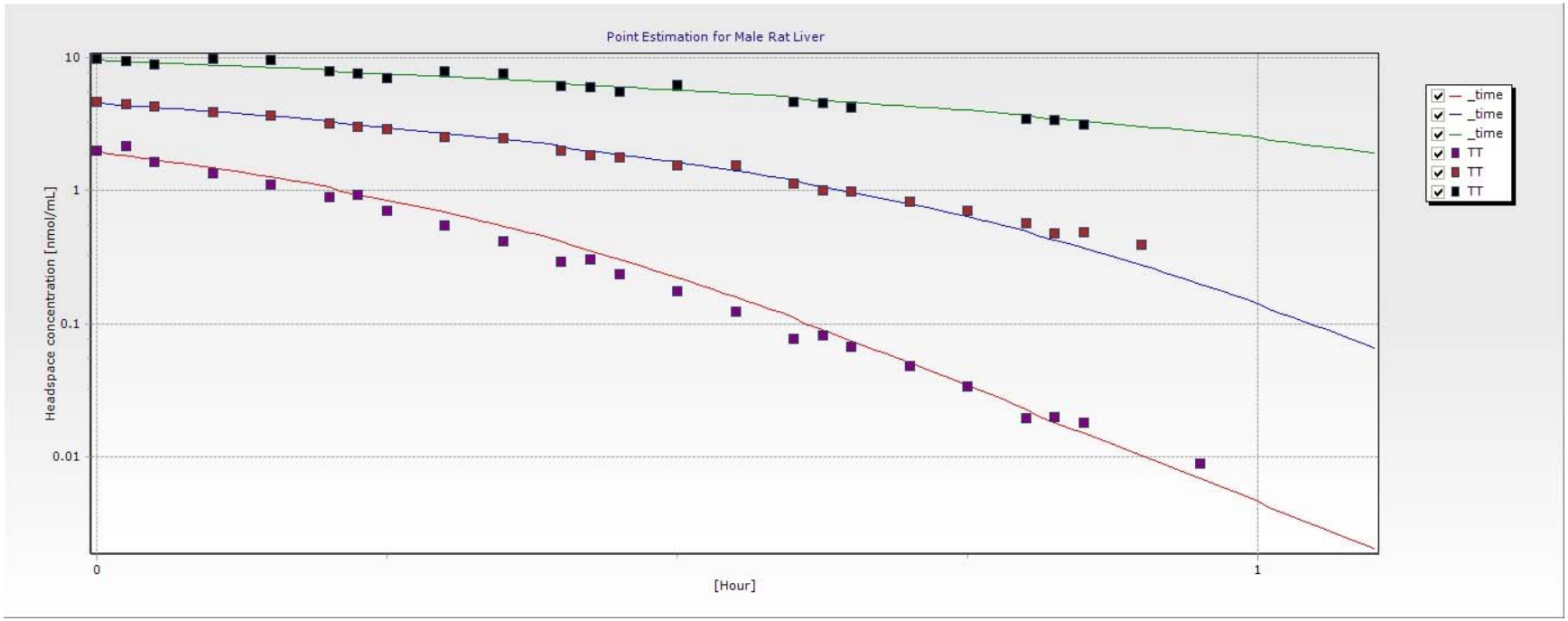
Note: Chloroprene headspace concentrations (symbols) and model simulations (lines) based on parameter values reported in Table 4.

Figure 7
Chloroprene oxidative metabolism time course in female B6C3F1 mouse kidney microsomes using point estimate model parameters



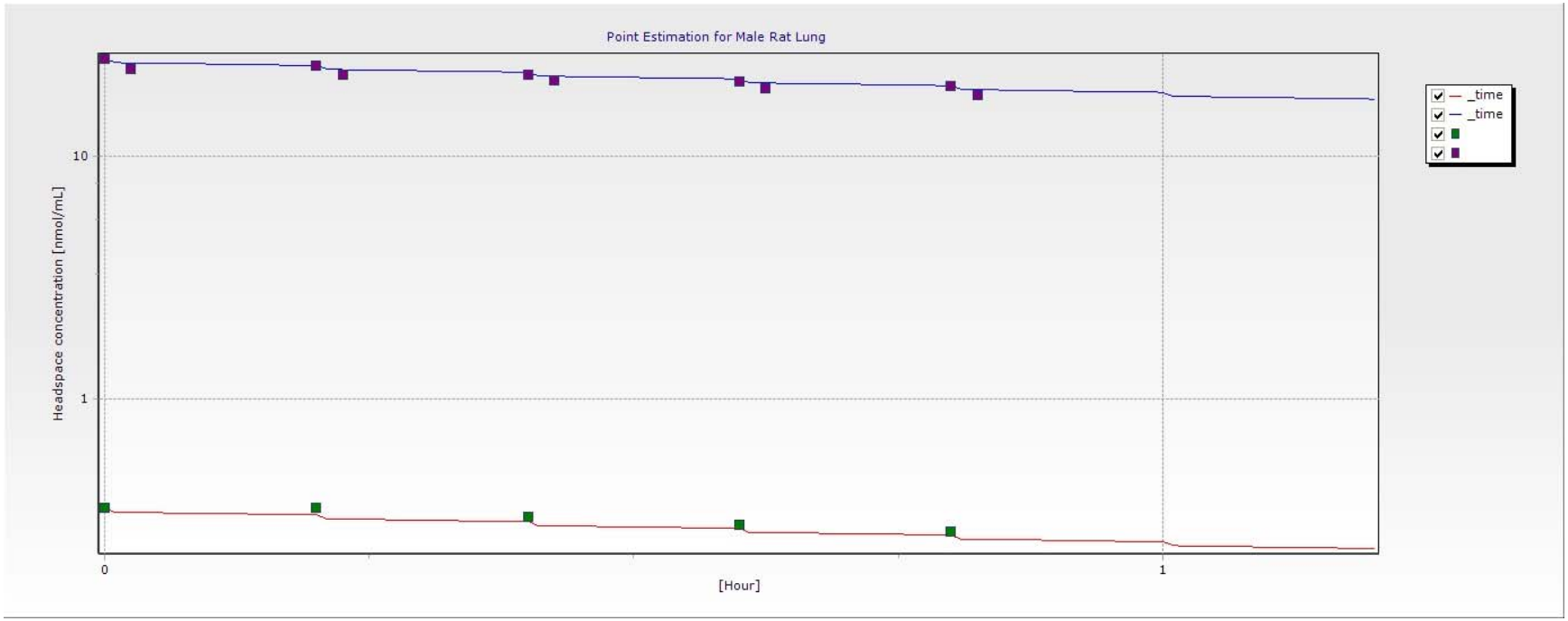
Note: Chloroprene headspace concentrations (symbols) and model simulations (lines) based on parameter values reported in Table 4.

Figure 8
Chloroprene oxidative metabolism time course in male Fischer rat liver microsomes using point estimate model parameters



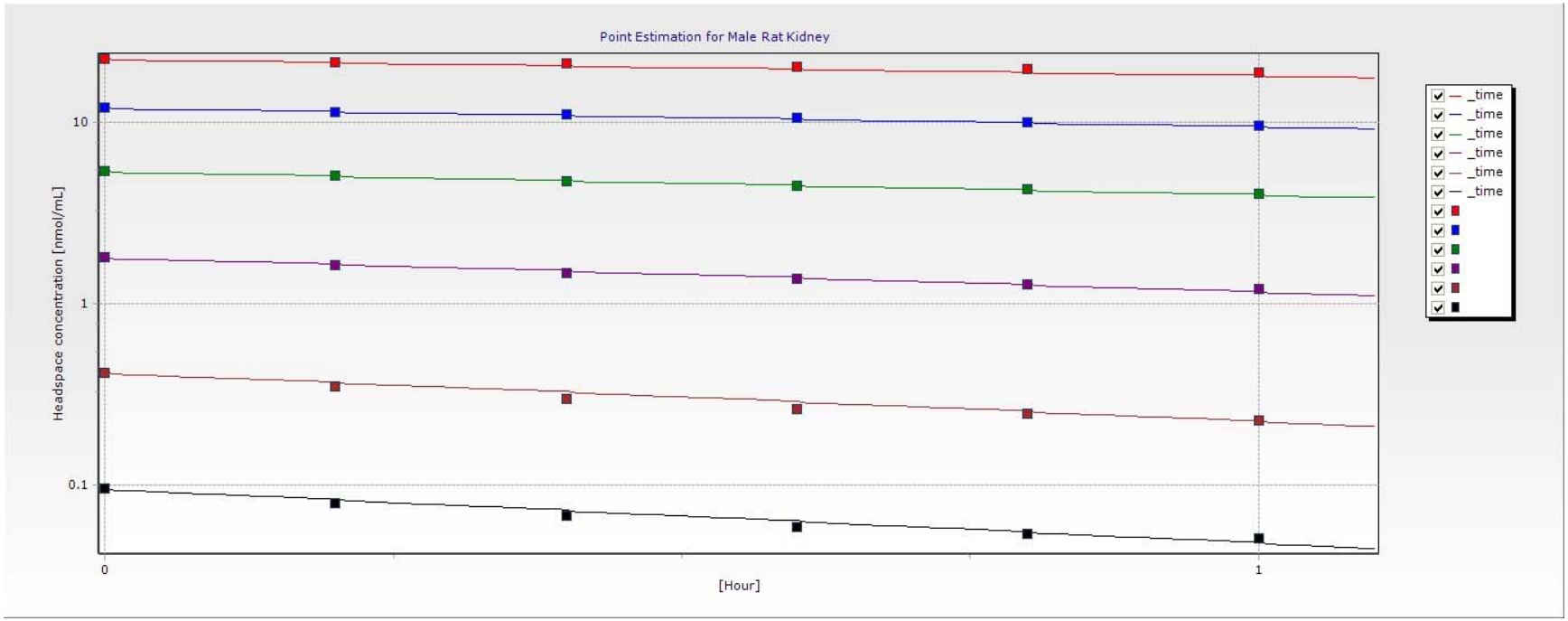
Note: Chloroprene headspace concentrations (symbols) and model simulations (lines) based on parameter values reported in Table 4.

Figure 9
Chloroprene oxidative metabolism time course in male Fischer rat lung microsomes using point estimate model parameters



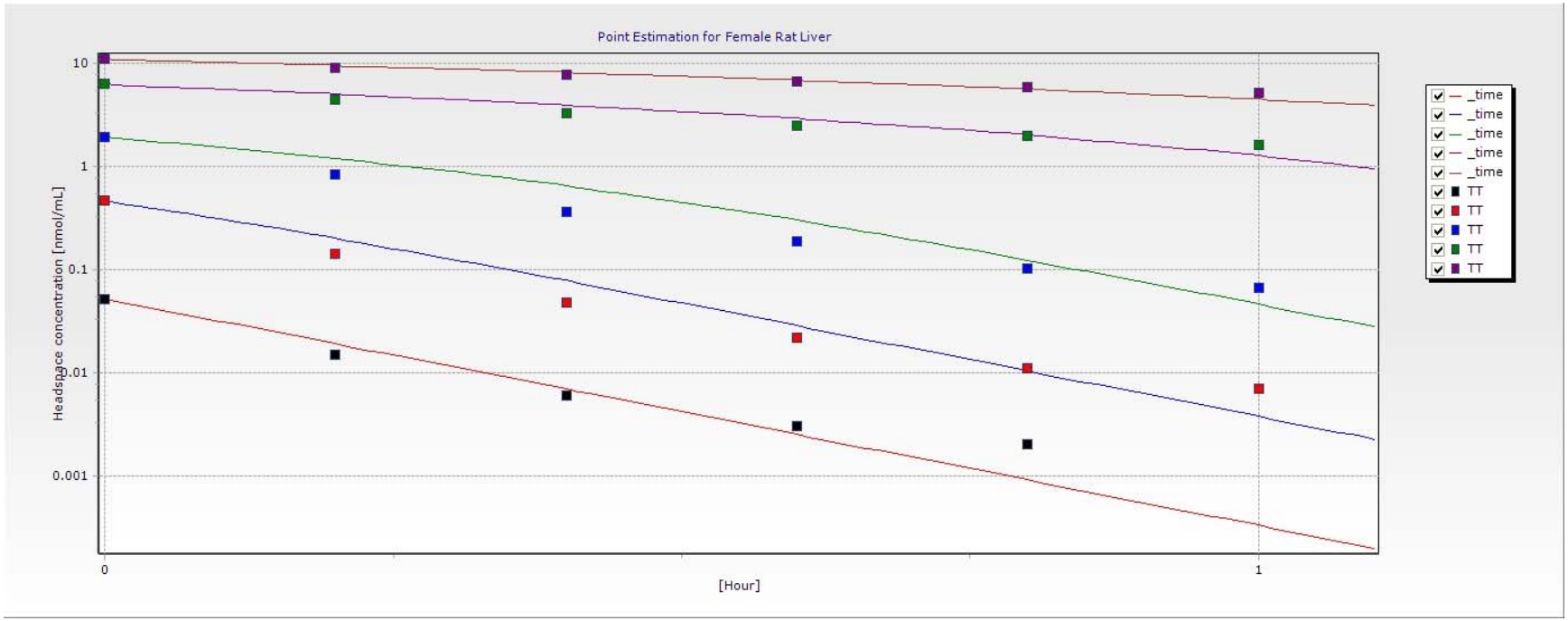
Note: Chloroprene headspace concentrations (symbols) and model simulations (lines) based on parameter values reported in Table 4.

Figure 10
Chloroprene oxidative metabolism time course in male Fischer rat kidney microsomes using point estimate model parameters



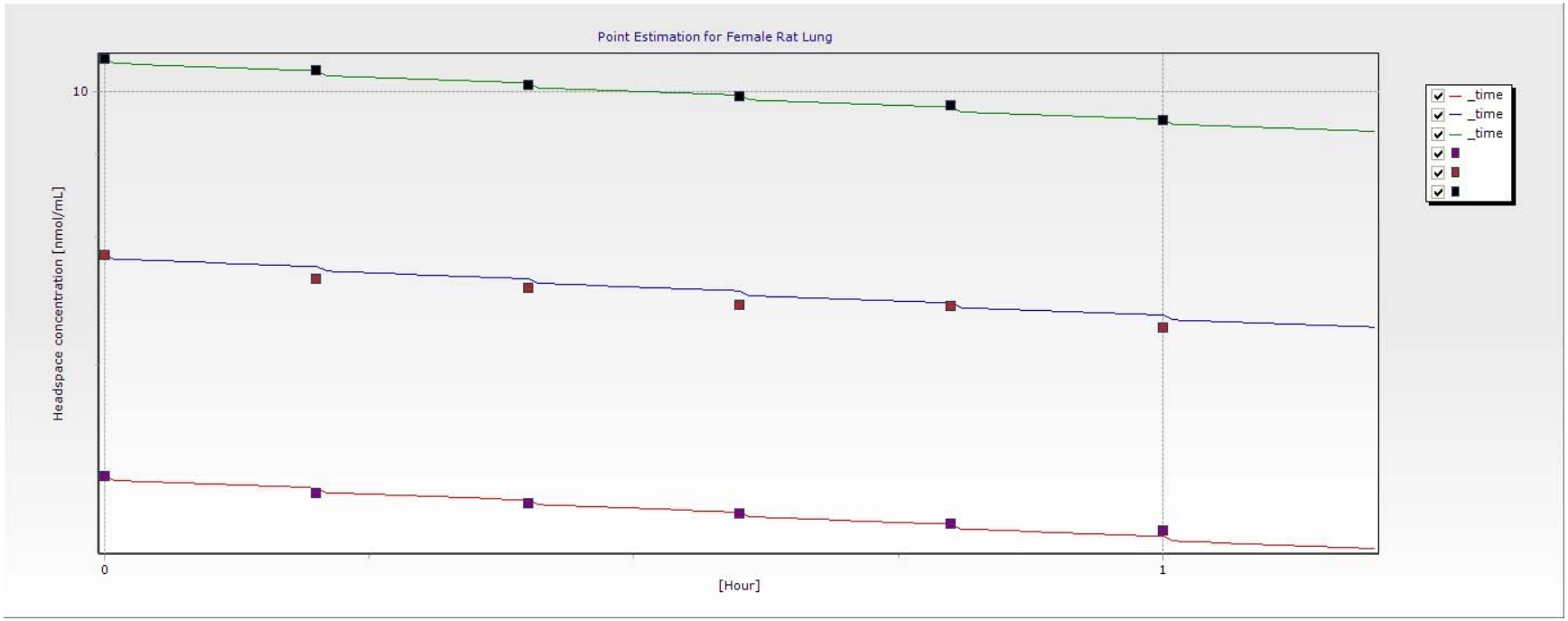
Note: Chloroprene headspace concentrations (symbols) and model simulations (lines) based on parameter values reported in Table 4.

Figure 11
Chloroprene oxidative metabolism time course in female Fischer rat liver microsomes using point estimate model parameters



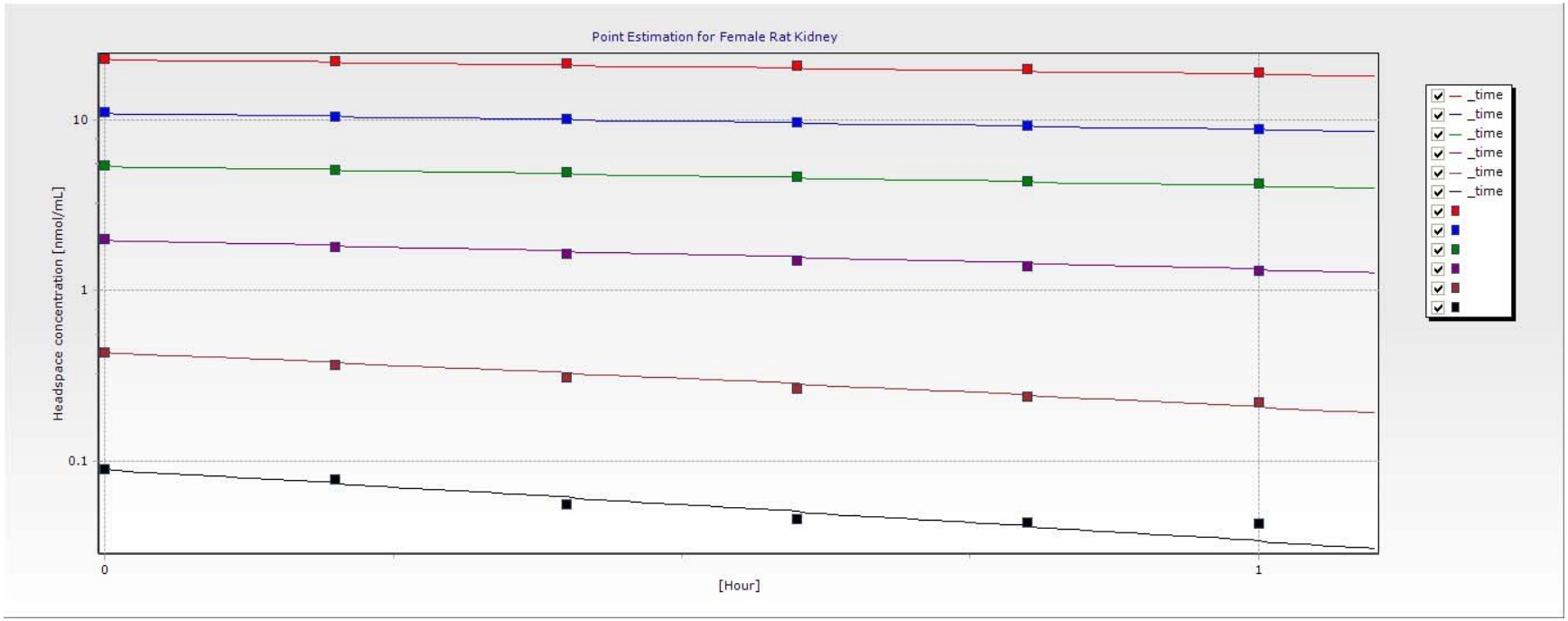
Note: Chloroprene headspace concentrations (symbols) and model simulations (lines) based on parameter values reported in Table 4.

Figure 12
Chloroprene oxidative metabolism time course in female Fischer rat lung microsomes using point estimate model parameters



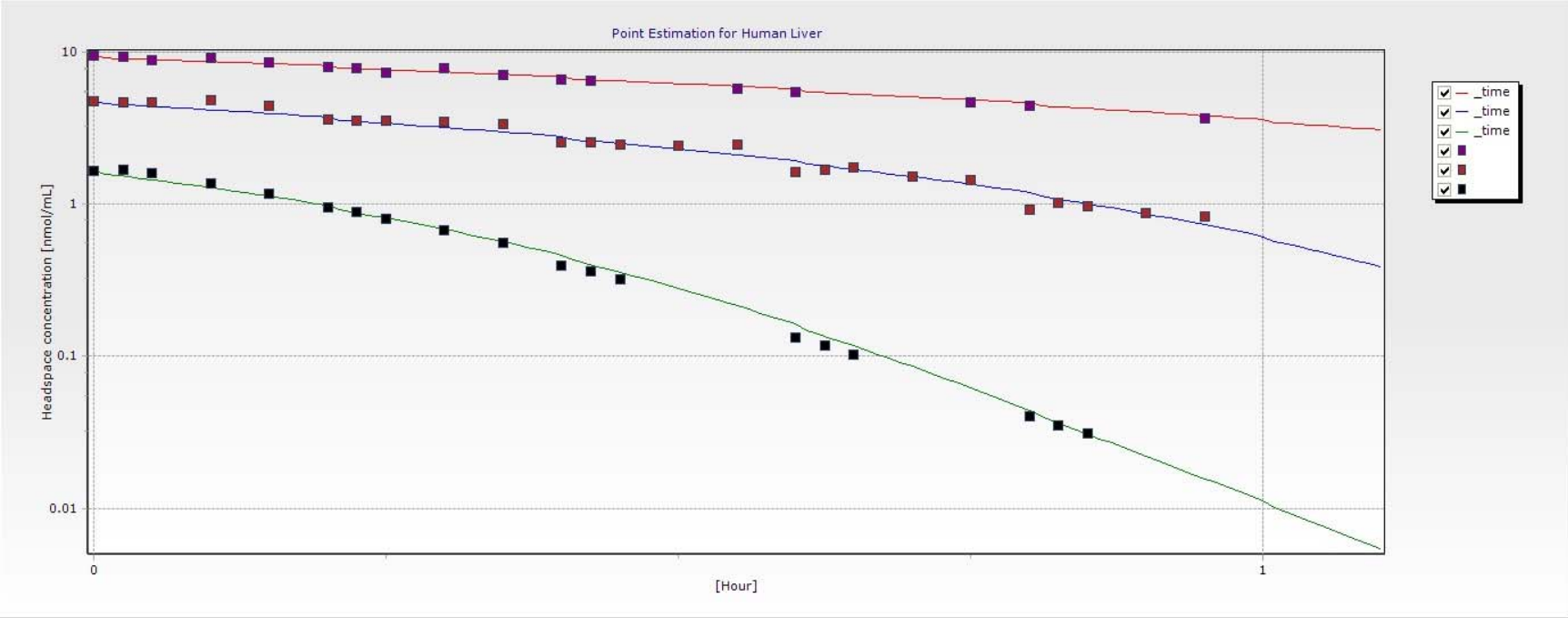
Note: Chloroprene headspace concentrations (symbols) and model simulations (lines) based on parameter values reported in Table 4.

Figure 13
Chloroprene oxidative metabolism time course in female Fischer rat kidney microsomes using point estimate model parameters



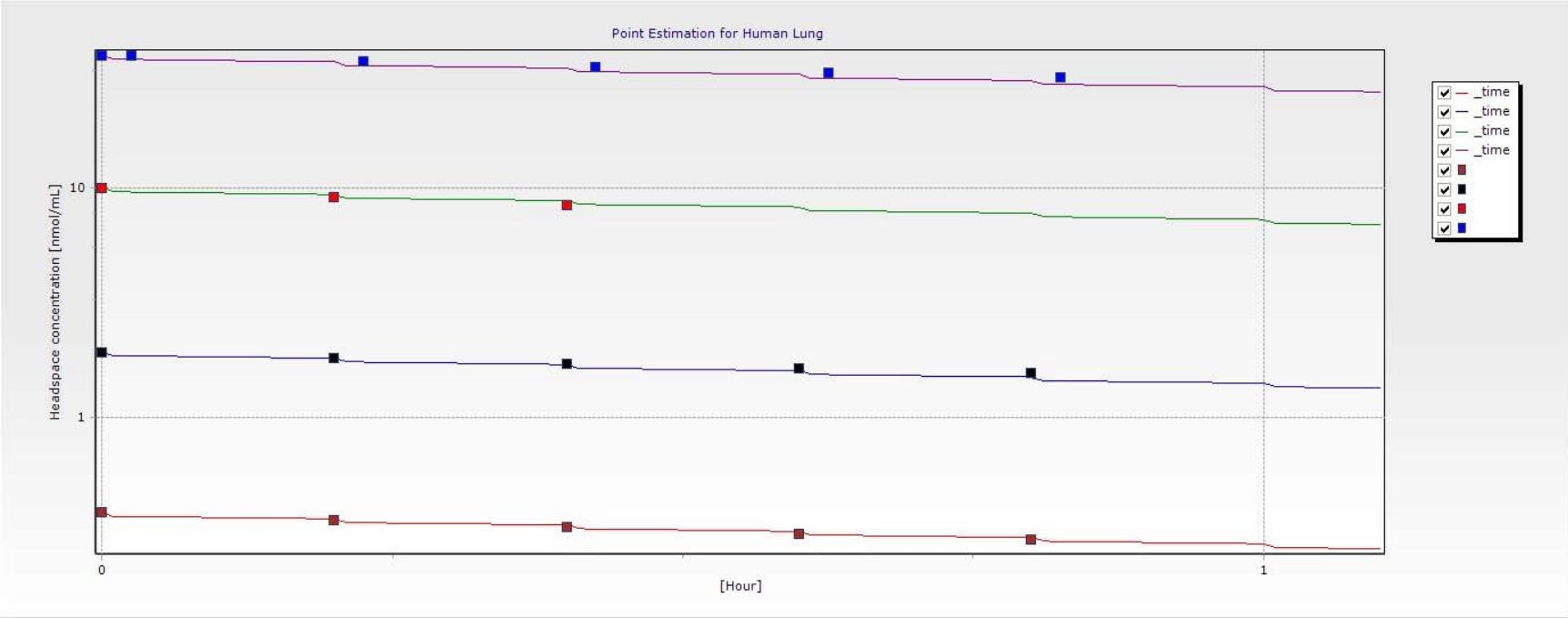
Note: Chloroprene headspace concentrations (symbols) and model simulations (lines) based on parameter values reported in Table 4.

Figure 14
Chloroprene oxidative metabolism time course in human (pooled mixed gender) liver microsomes using point estimate model parameters



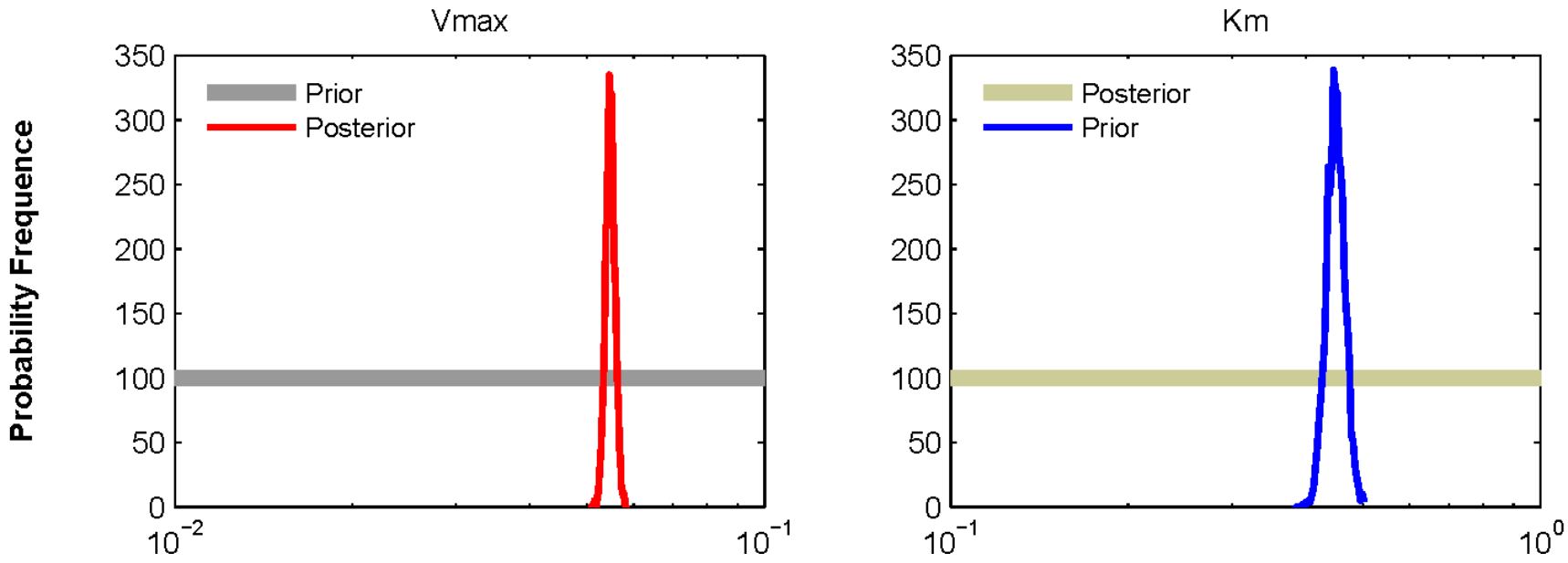
Note: Chloroprene headspace concentrations (symbols) and model simulations (lines) based on parameter values reported in Table 4.

Figure 15
Chloroprene oxidative metabolism time course in human (pooled mixed gender) lung microsomes using point estimate model parameters



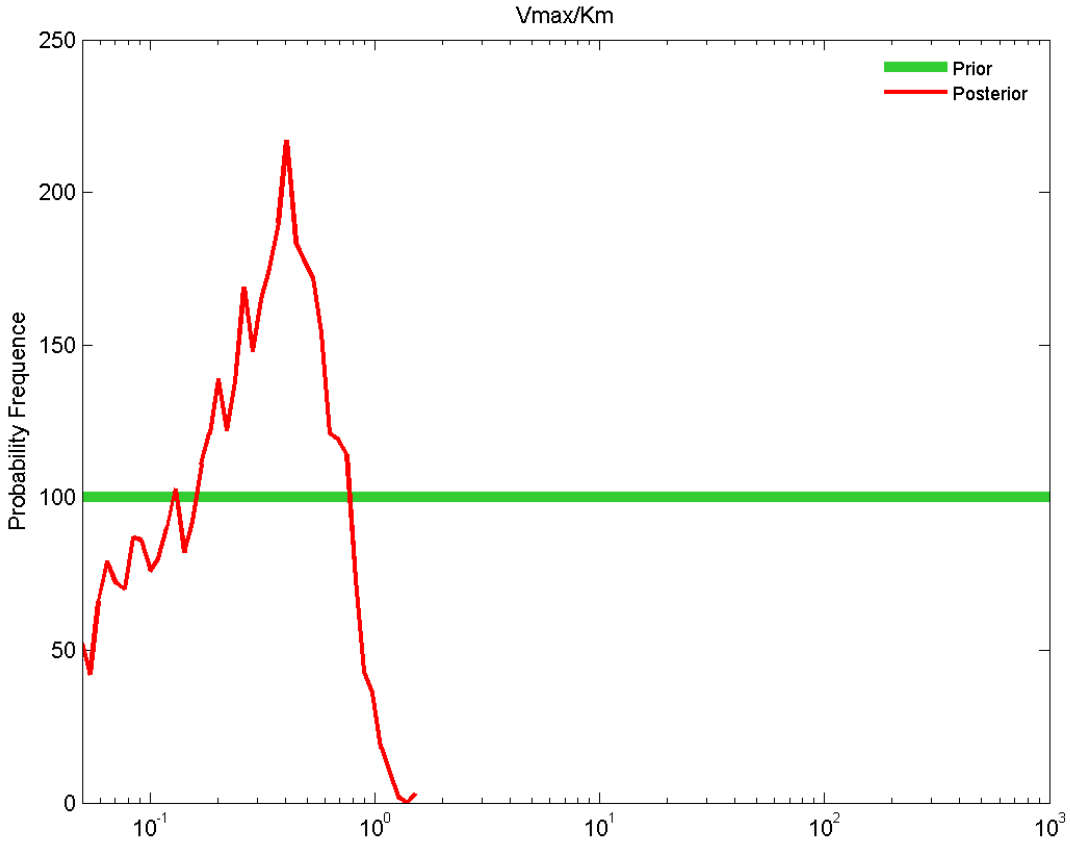
Note: Chloroprene headspace concentrations (symbols) and model simulations (lines) based on parameter values reported in Table 4.

Figure 16
Representative comparison of uniform prior and posterior distributions for human (pooled mixed gender) liver microsomal metabolism parameters



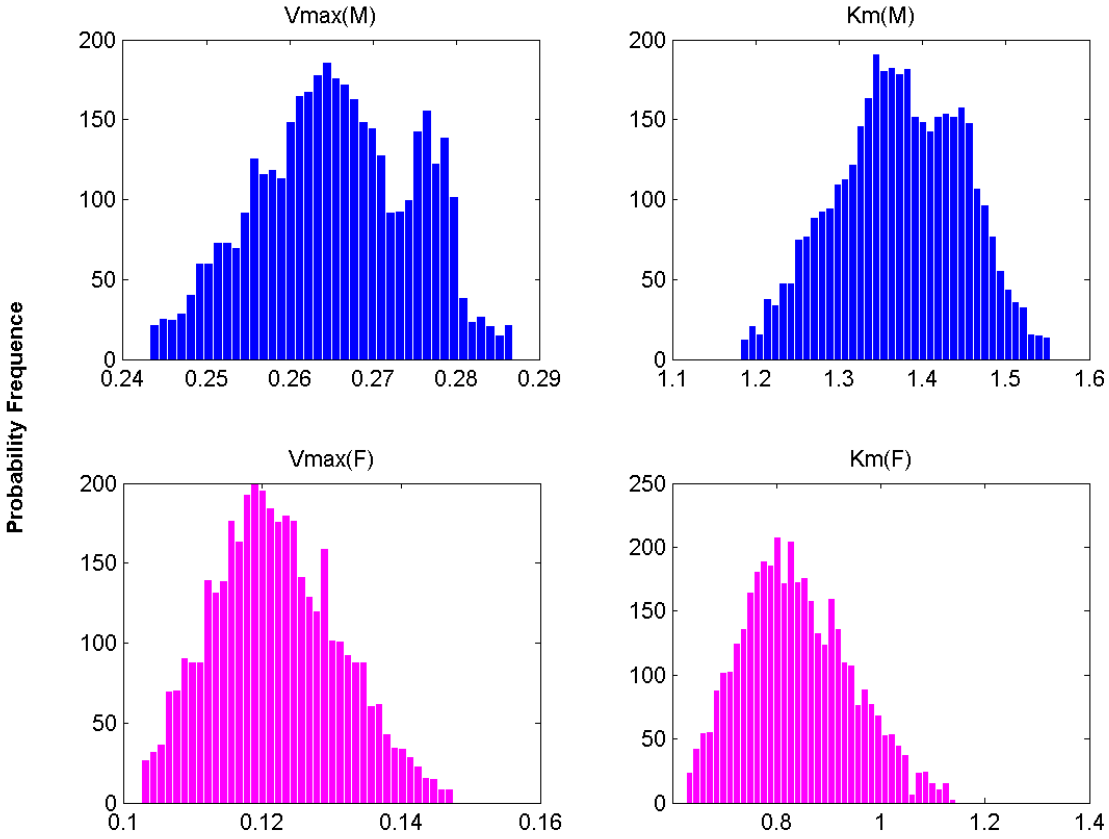
Note: Vmax (µmol/hr/mg microsomal protein) or Km (µmol/L) posterior frequency counts (per 4000 simulations).

Figure 17
Representative comparison of uniform prior and posterior distributions for oxidative metabolism of chloroprene in human (pooled mixed gender) lung microsomes



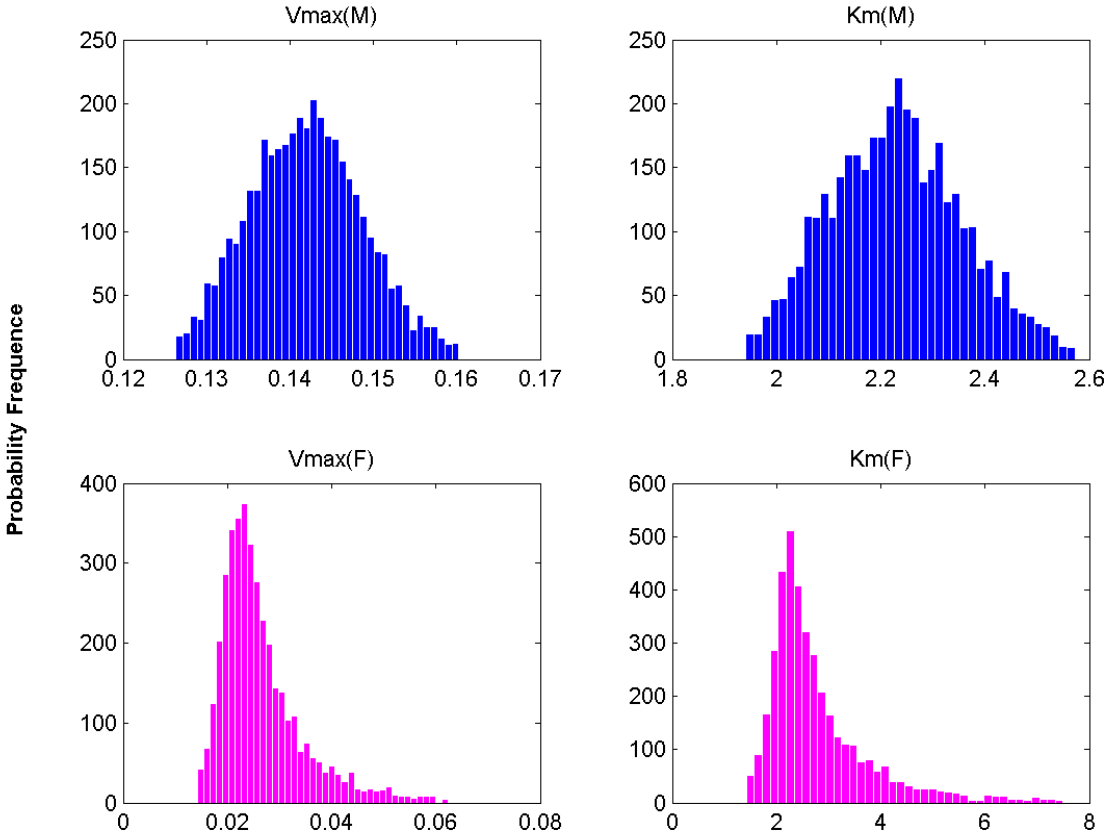
Note: Vmax/Km (L/hr/g microsomal protein) posterior frequency counts (per 4000 simulations).

Figure 18
Probability frequency of chloroprene oxidative metabolism parameters in male (M) and female (F) B6C3F1 mouse liver microsomes



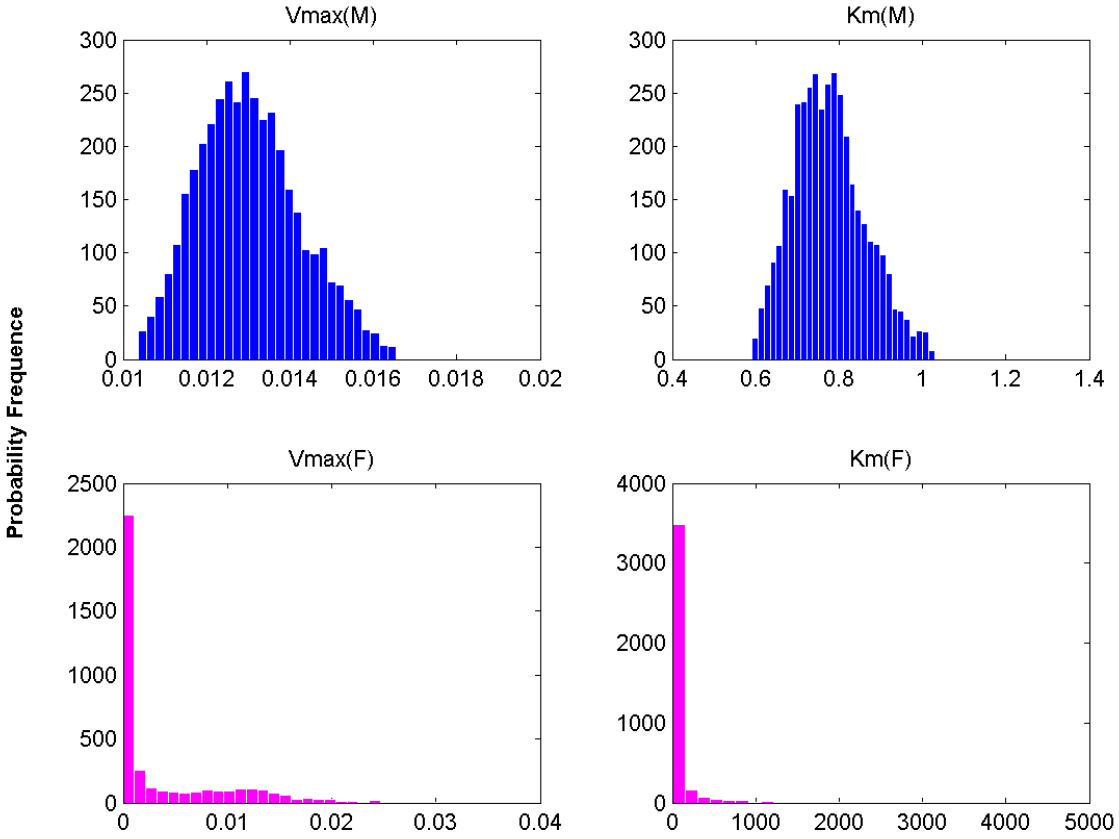
Note: Vmax (µmol/hr/mg microsomal protein) or Km (µmol/L) posterior frequency counts (per 4000 simulations).

Figure 19
Probability frequency of chloroprene oxidative metabolism parameters in male (M) and female (F) B6C3F1 mouse lung microsomes



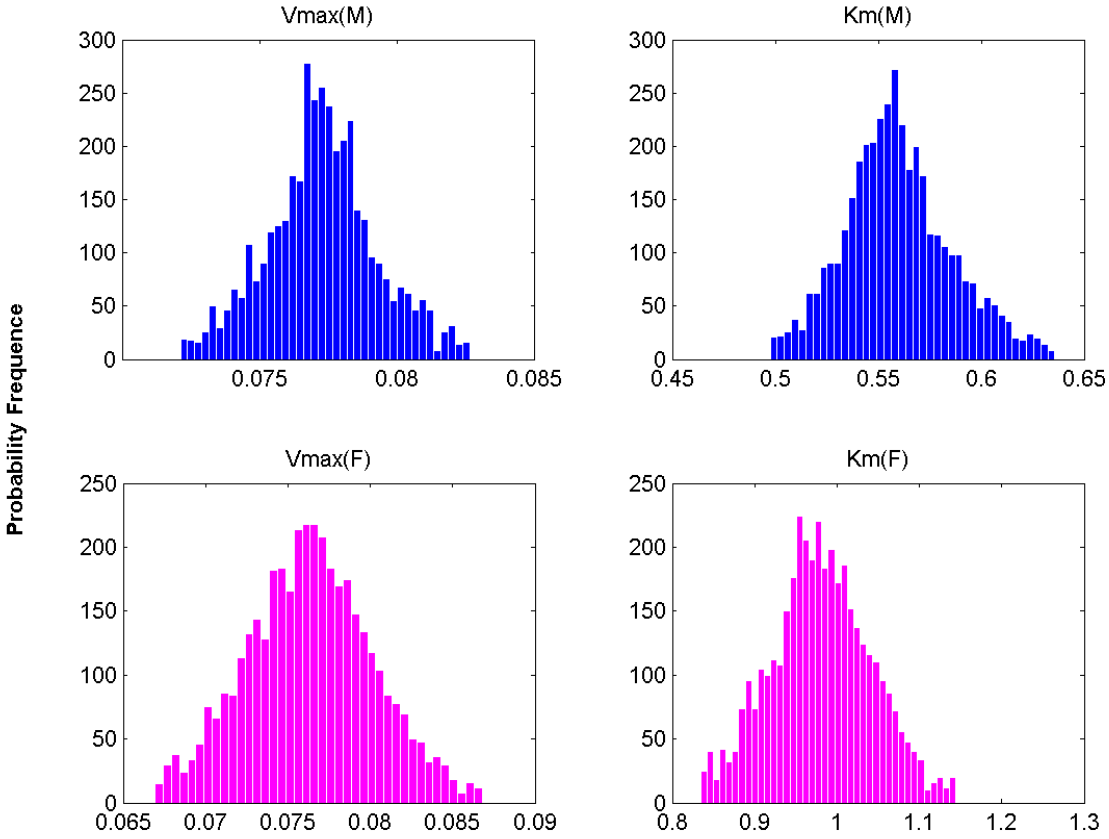
Note: Vmax (µmol/hr/mg microsomal protein) or Km (µmol/L) posterior frequency counts (per 4000 simulations).

Figure 20
Probability frequency of chloroprene oxidative metabolism parameters in male (M) and female (F) B6C3F1 mouse kidney microsomes



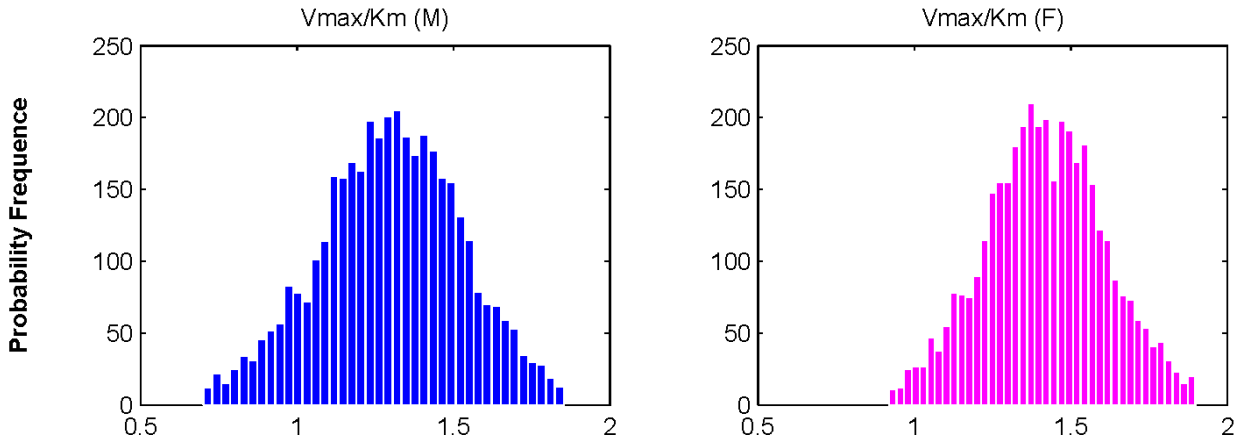
Note: Vmax (µmol/hr/mg microsomal protein) or Km (µmol/L) posterior frequency counts (per 4000 simulations).

Figure 21
Probability frequency of chloroprene oxidative metabolism parameters in male (M) and female (F) Fischer rat liver microsomes



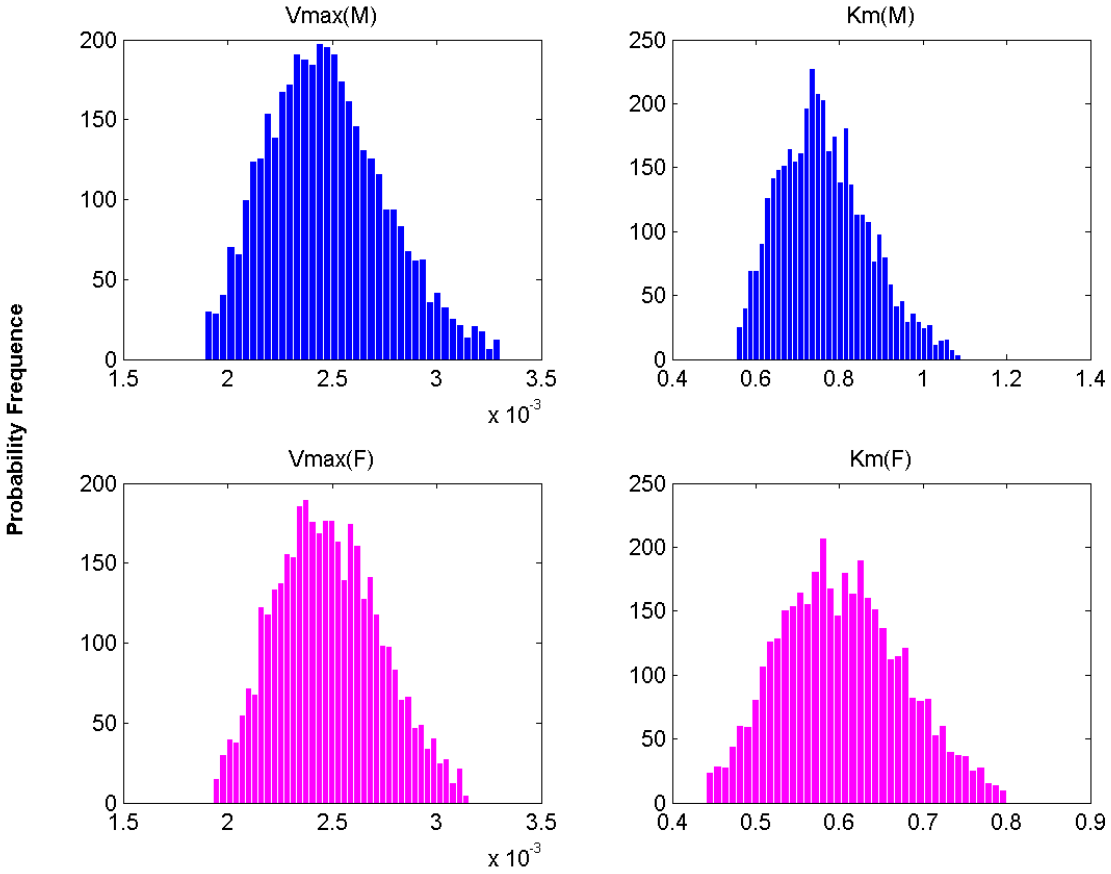
Note: Vmax (µmol/hr/mg microsomal protein) or Km (µmol/L) posterior frequency counts (per 4000 simulations).

Figure 22
Probability frequency of chloroprene oxidative metabolism parameters in male (M) and female (F) Fischer rat lung microsomes



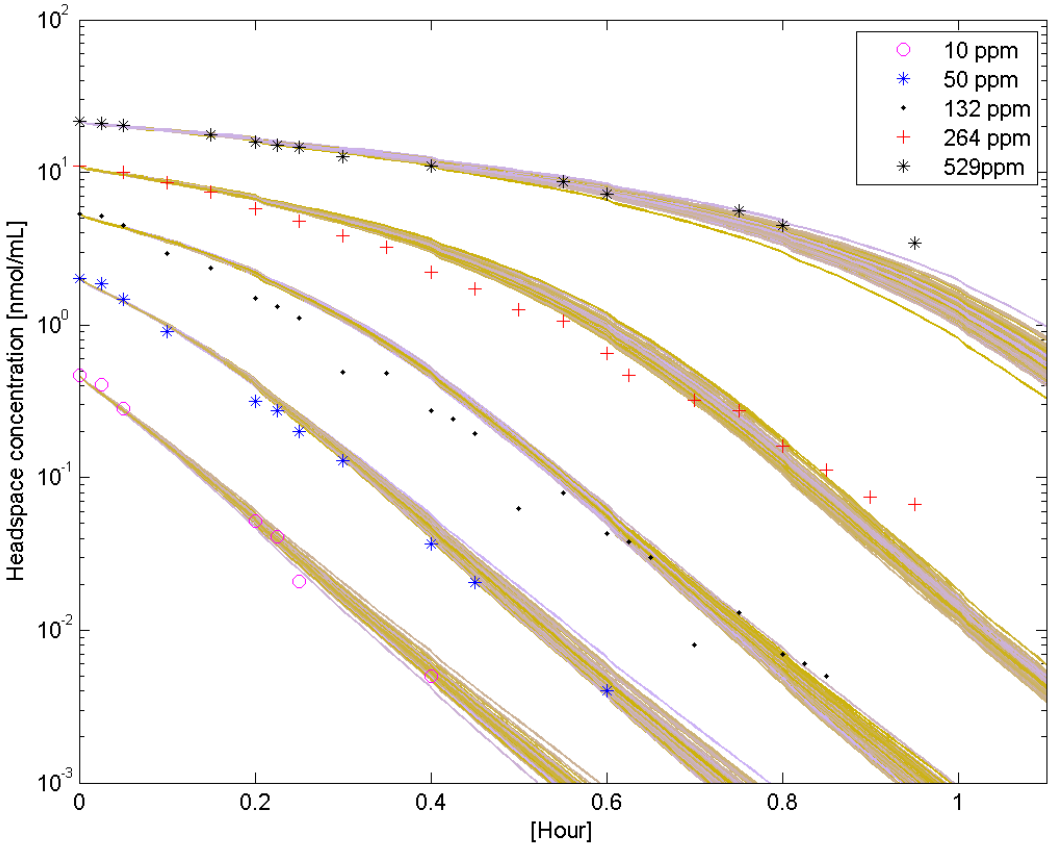
Note: Vmax/Km (L/hr/g microsomal protein) posterior frequency counts (per 4000 simulations).

Figure 23
Probability frequency of chloroprene oxidative metabolism parameters in male (M) and female (F) Fischer rat kidney microsomes



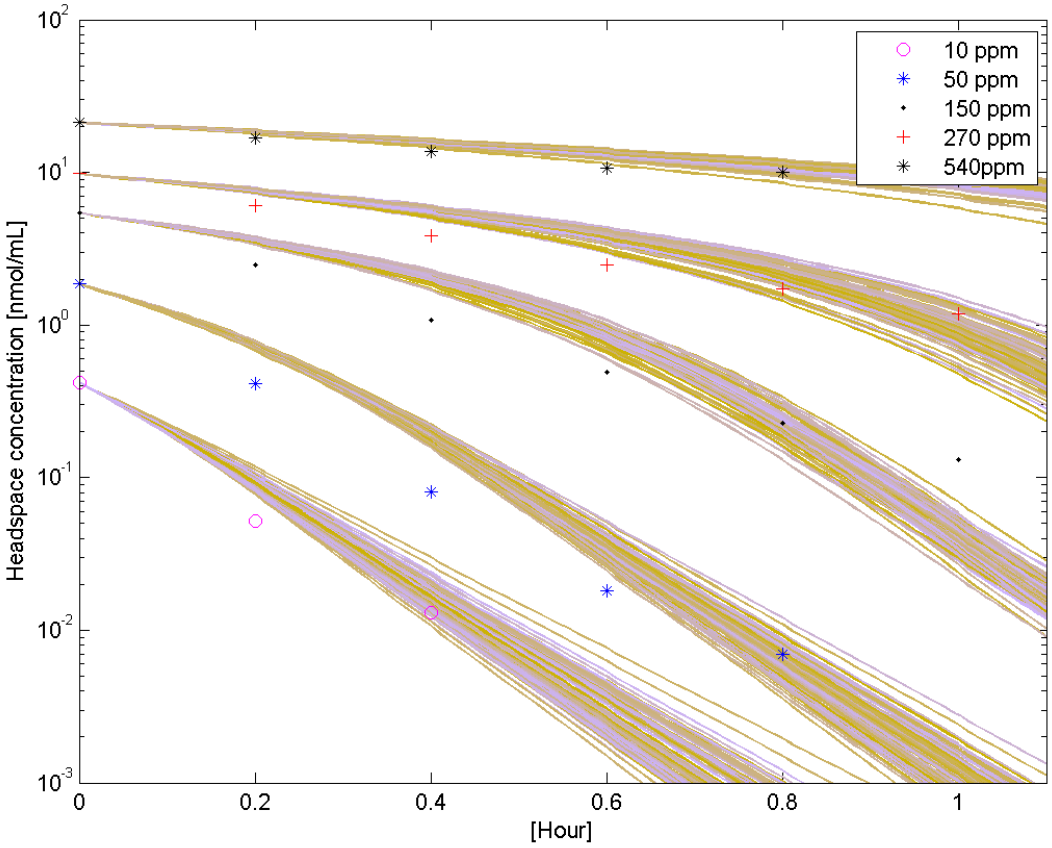
Note: Vmax (µmol/hr/mg microsomal protein) or Km (µmol/L) posterior frequency counts (per 4000 simulations).

Figure 24
Distribution of chloroprene oxidative metabolism time course in male B6C3F1 mouse liver microsomes



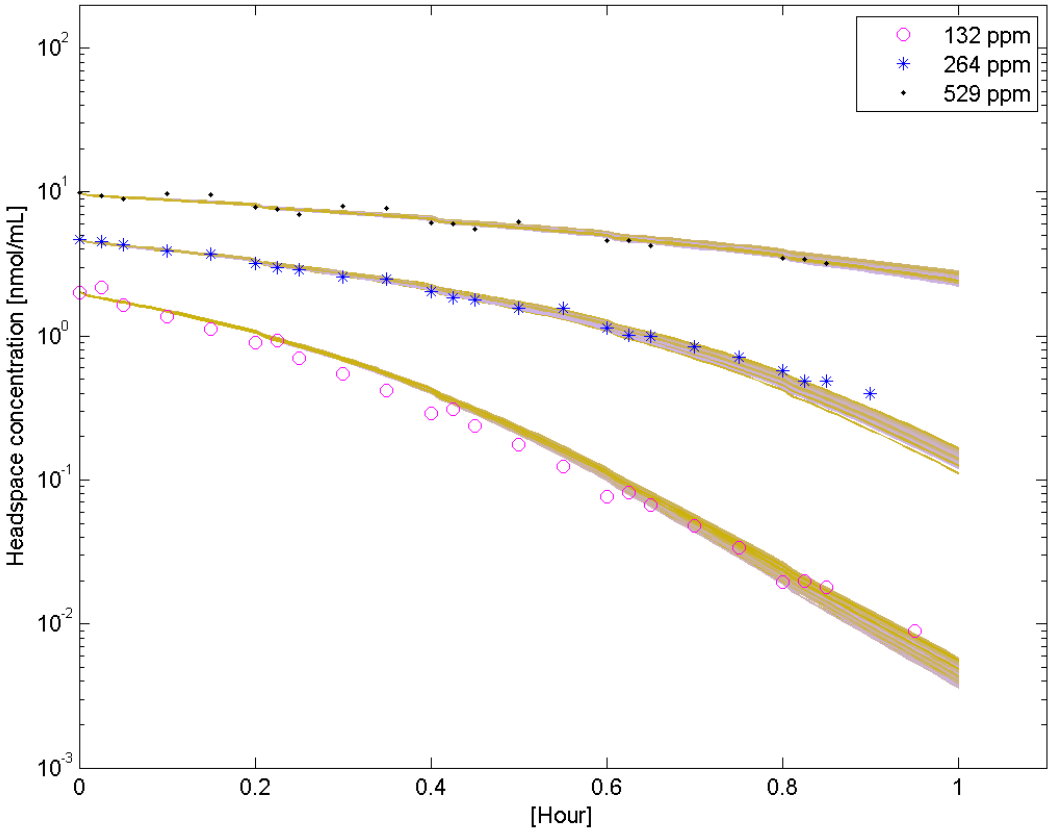
Note: Chloroprene headspace concentrations (symbols) and model simulations (50 lines) based on posterior distribution for parameter values as reported in Table 5. Simulations for each starting concentration represent 50 sets of model parameters randomly drawn from the posterior distributions.

Figure 25
Distribution of chloroprene oxidative metabolism time course in female B6C3F1 mouse liver microsomes



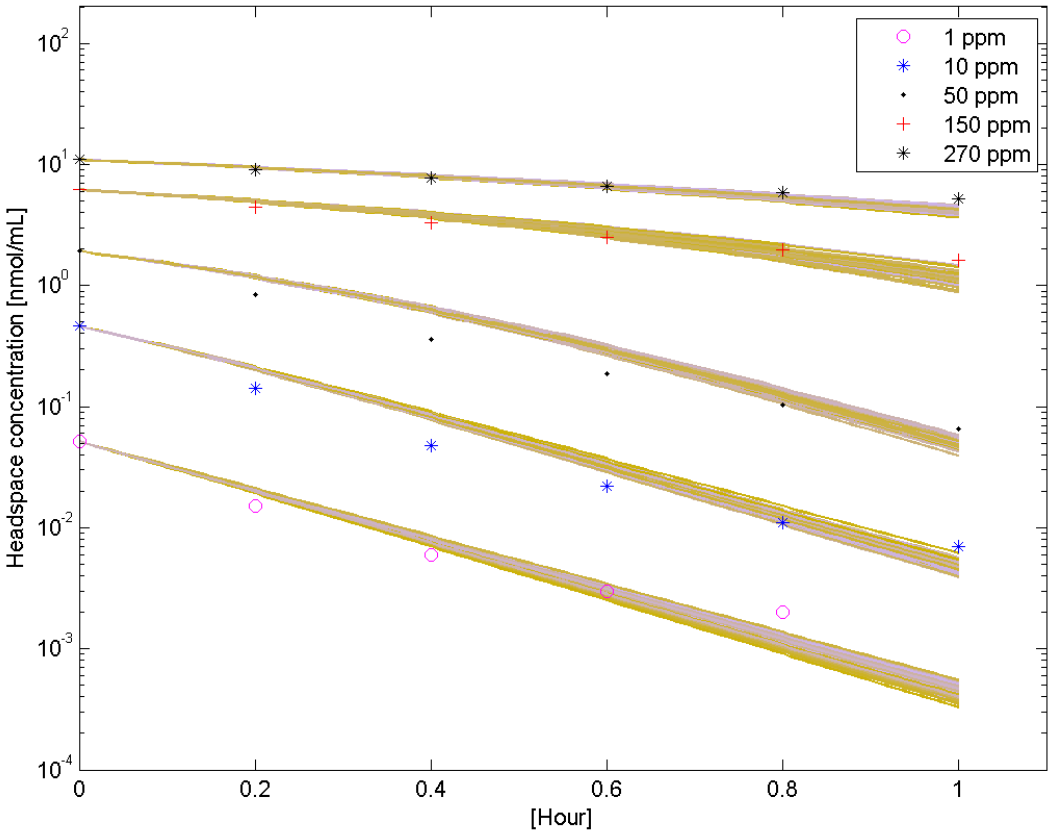
Note: Chloroprene headspace concentrations (symbols) and model simulations (50 lines) based on posterior distribution for parameter values as reported in Table 5. Simulations for each starting concentration represent 50 sets of model parameters randomly drawn from the posterior distributions.

Figure 26
Distribution of chloroprene oxidative metabolism time course in male Fischer rat liver microsomes



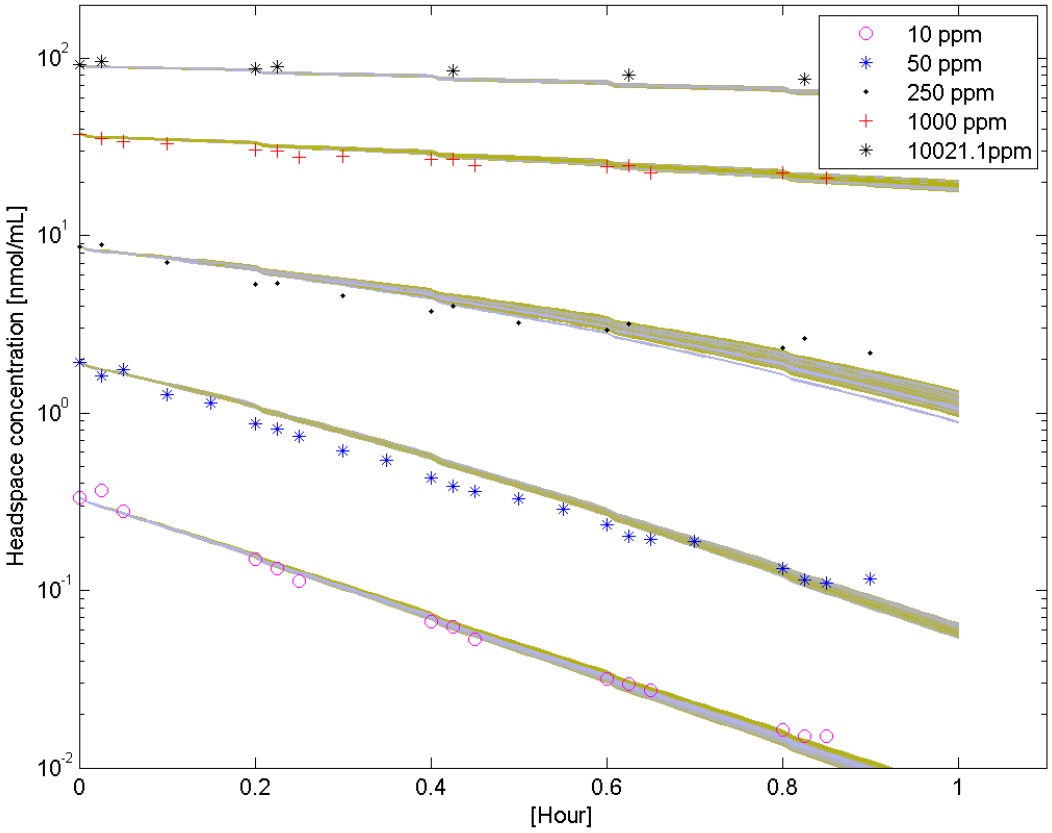
Note: Chloroprene headspace concentrations (symbols) and model simulations (50 lines) based on posterior distribution for parameter values as reported in Table 5. Simulations for each starting concentration represent 50 sets of model parameters randomly drawn from the posterior distributions.

Figure 27
Distribution of chloroprene oxidative metabolism time course in female Fischer rat liver microsomes



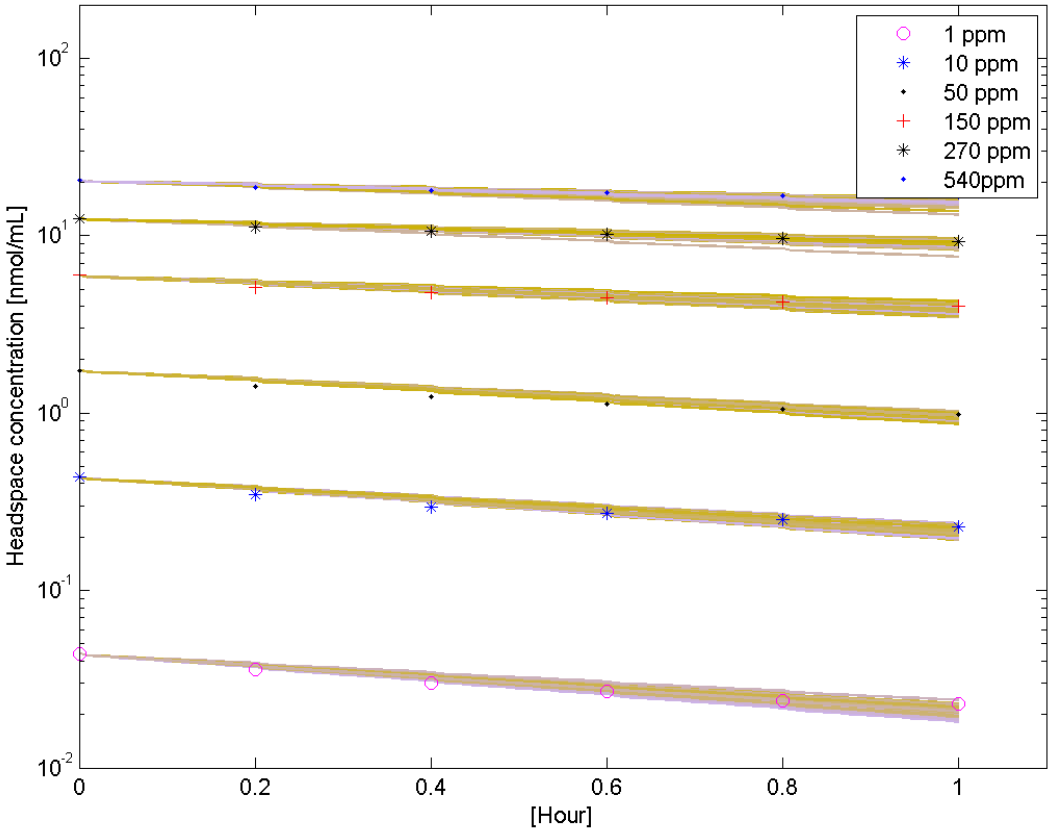
Note: Chloroprene headspace concentrations (symbols) and model simulations (50 lines) based on posterior distribution for parameter values as reported in Table 5. Simulations for each starting concentration represent 50 sets of model parameters randomly drawn from the posterior distributions.

Figure 28
Distribution of chloroprene oxidative metabolism time course in male B6C3F1 mouse lung microsomes



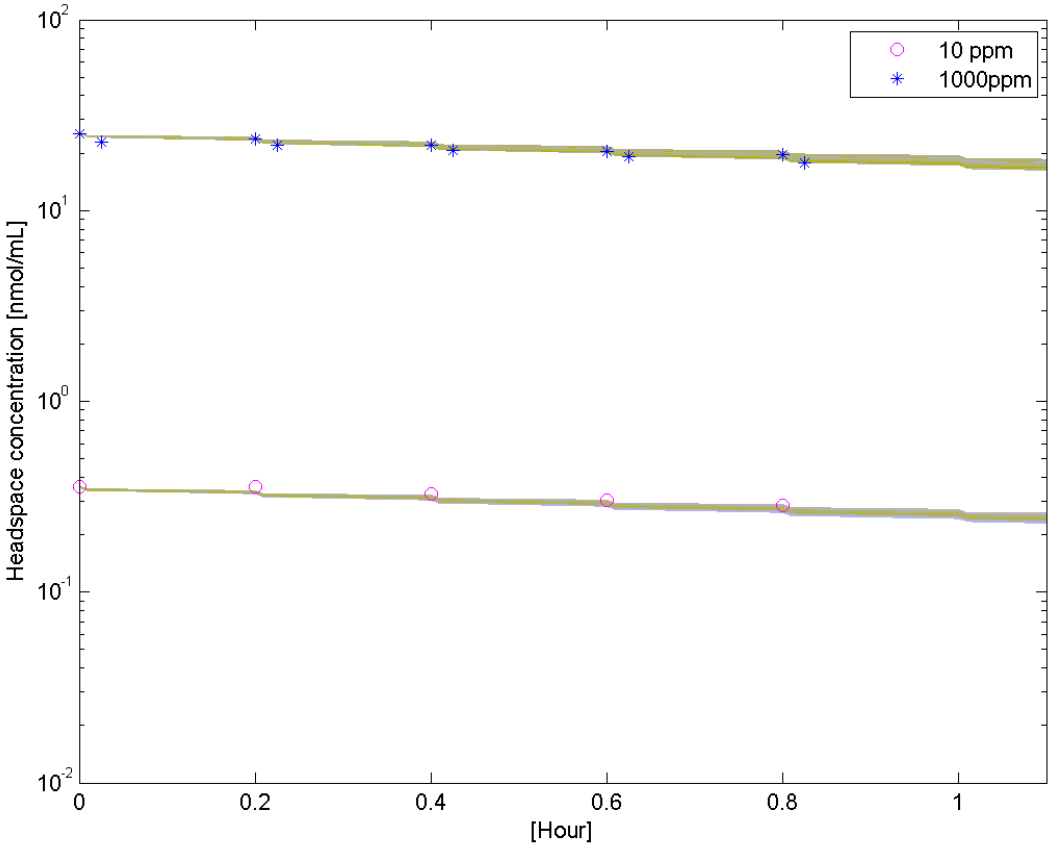
Note: Chloroprene headspace concentrations (symbols) and model simulations (50 lines) based on posterior distribution for parameter values as reported in Table 5. Simulations for each starting concentration represent 50 sets of model parameters randomly drawn from the posterior distributions.

Figure 29
Distribution of chloroprene oxidative metabolism time course in female B6C3F1 mouse lung microsomes



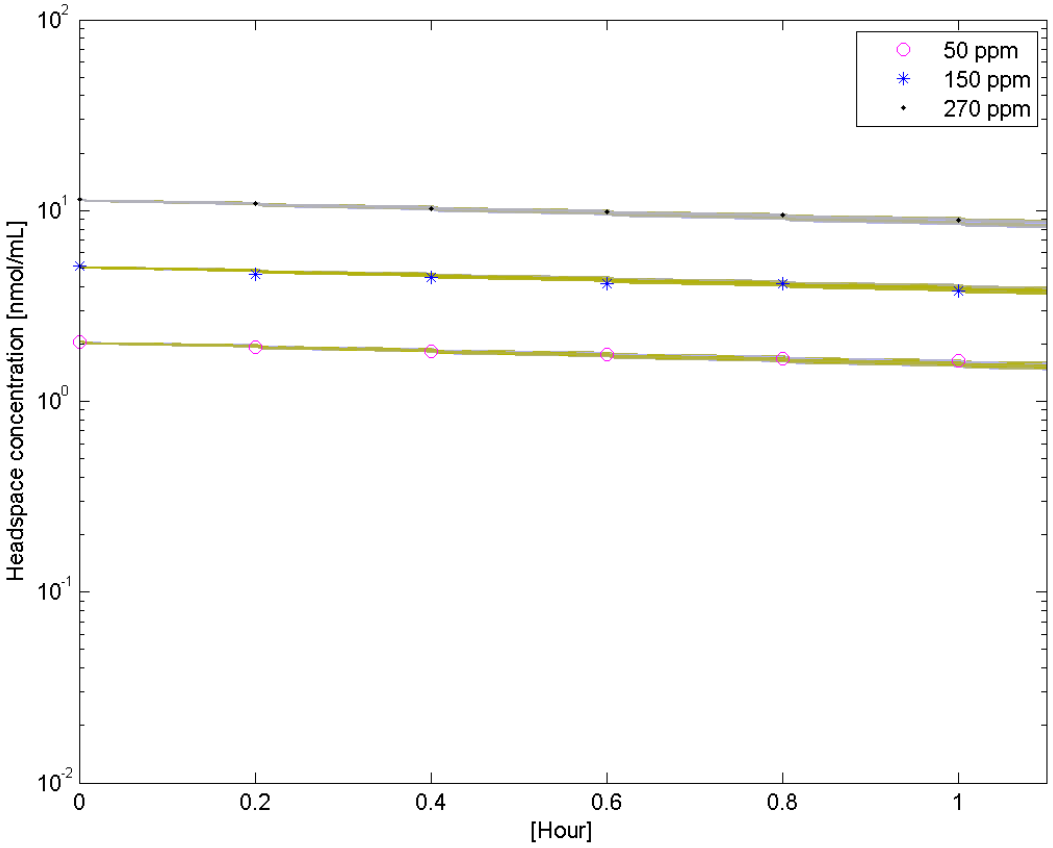
Note: Chloroprene headspace concentrations (symbols) and model simulations (50 lines) based on posterior distribution for parameter values as reported in Table 5. Simulations for each starting concentration represent 50 sets of model parameters randomly drawn from the posterior distributions.

Figure 30
Distribution of chloroprene oxidative metabolism time course in male Fischer rat lung microsomes



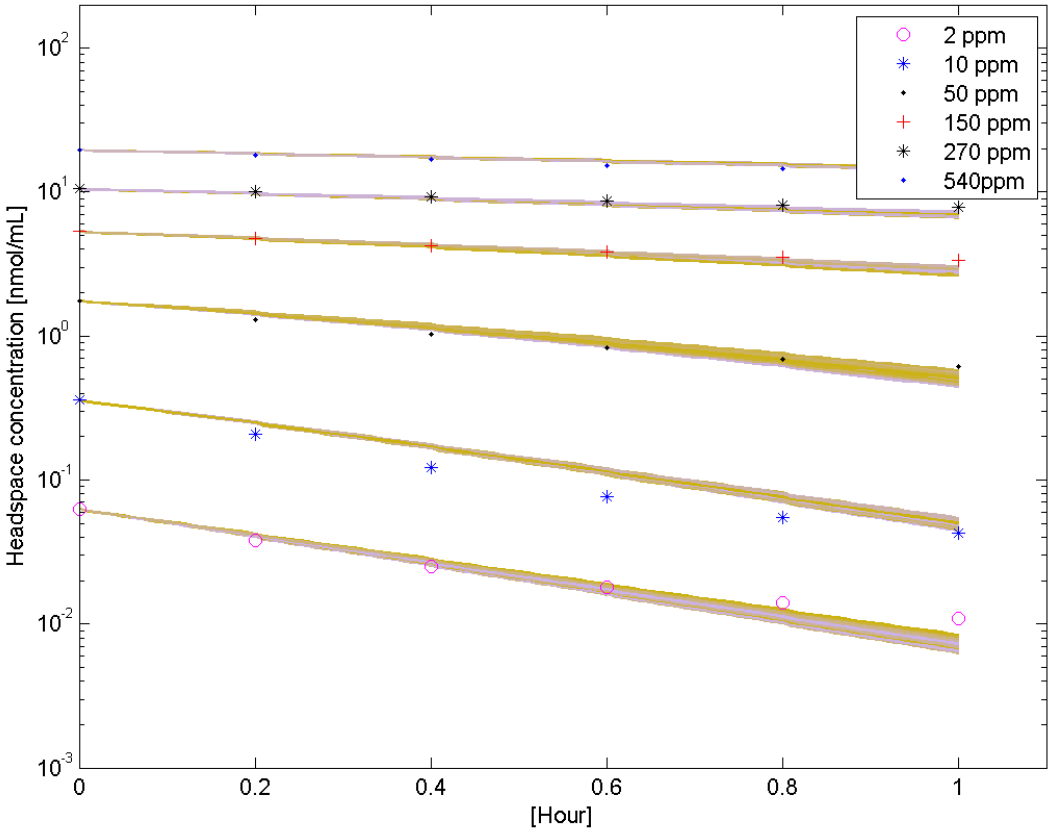
Note: Chloroprene headspace concentrations (symbols) and model simulations (50 lines) based on posterior distribution for parameter values as reported in Table 5. Simulations for each starting concentration represent 50 sets of model parameters randomly drawn from the posterior distributions.

Figure 31
Distribution of chloroprene oxidative metabolism time course in female Fischer rat lung microsomes



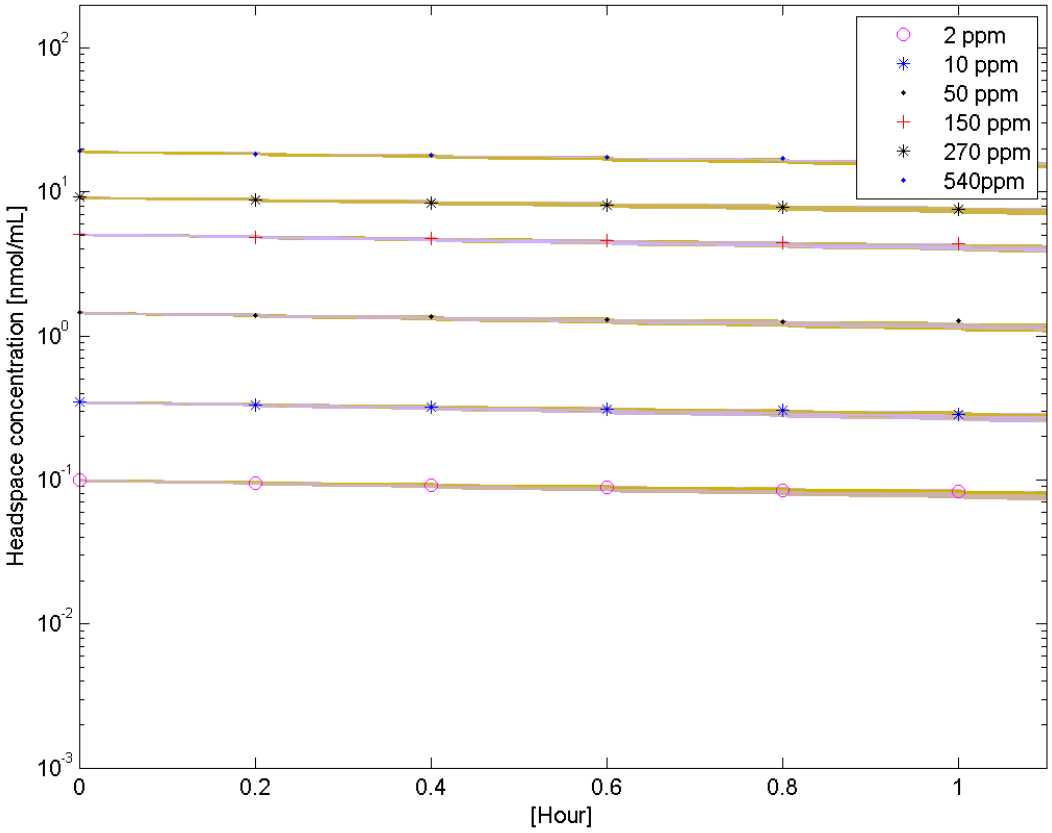
Note: Chloroprene headspace concentrations (symbols) and model simulations (50 lines) based on posterior distribution for parameter values as reported in Table 5. Simulations for each starting concentration represent 50 sets of model parameters randomly drawn from the posterior distributions.

Figure 32
Distribution of chloroprene oxidative metabolism time course in male B6C3F1 mouse kidney microsomes



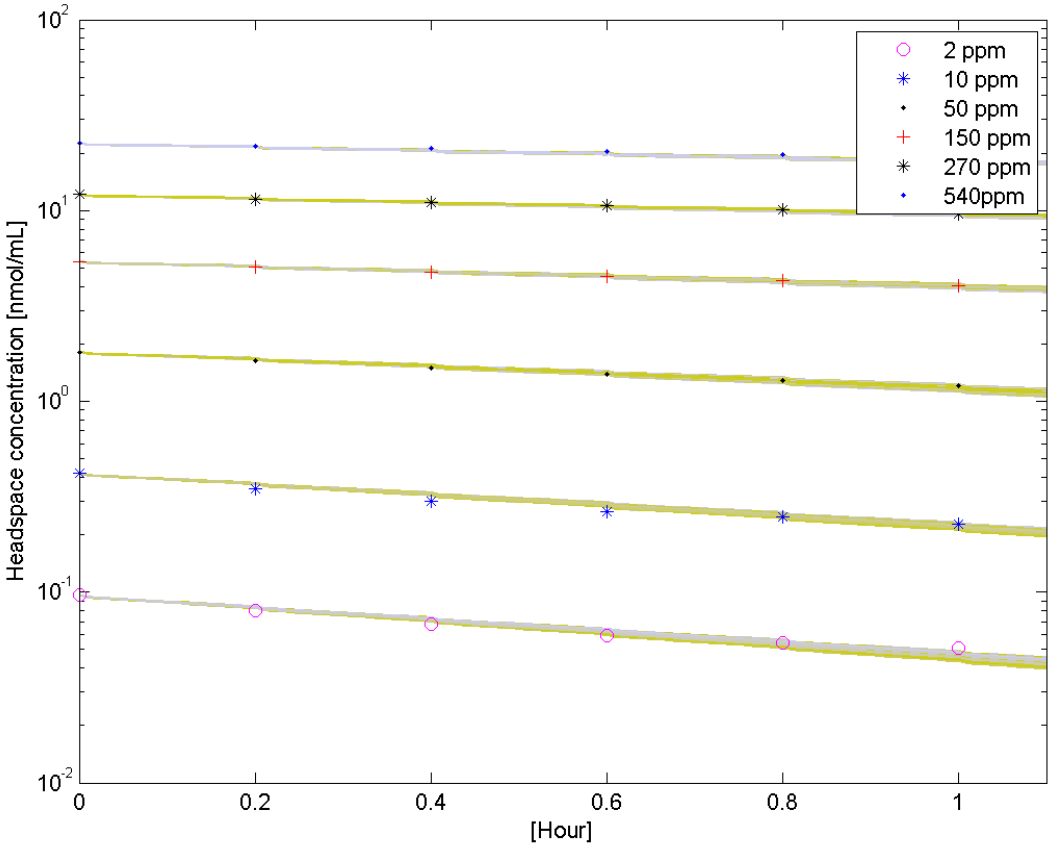
Note: Chloroprene headspace concentrations (symbols) and model simulations (50 lines) based on posterior distribution for parameter values as reported in Table 5. Simulations for each starting concentration represent 50 sets of model parameters randomly drawn from the posterior distributions.

Figure 33
Distribution of chloroprene oxidative metabolism time course in female B6C3F1 mouse kidney microsomes



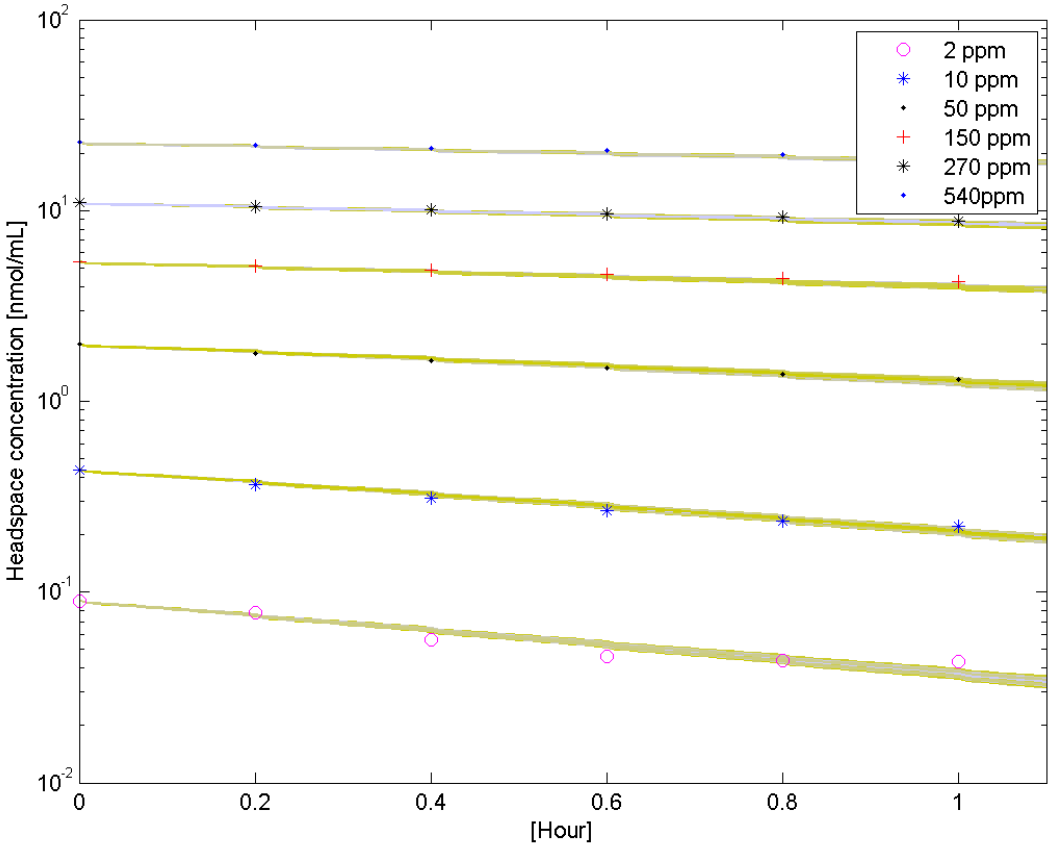
Note: Chloroprene headspace concentrations (symbols) and model simulations (50 lines) based on posterior distribution for parameter values as reported in Table 5. Simulations for each starting concentration represent 50 sets of model parameters randomly drawn from the posterior distributions.

Figure 34
Distribution of chloroprene oxidative metabolism time course in male Fischer rat kidney microsomes



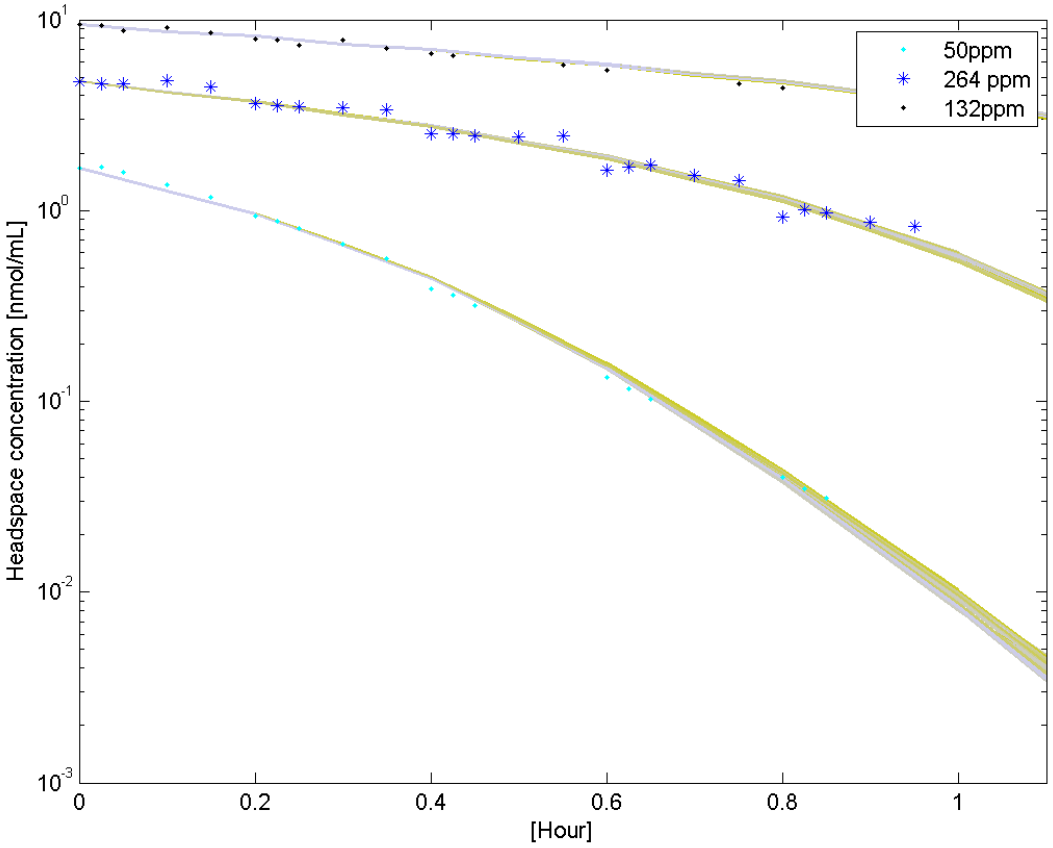
Note: Chloroprene headspace concentrations (symbols) and model simulations (50 lines) based on posterior distribution for parameter values as reported in Table 5. Simulations for each starting concentration represent 50 sets of model parameters randomly drawn from the posterior distributions.

Figure 35
Distribution of chloroprene oxidative metabolism time course in male Fischer rat kidney microsomes



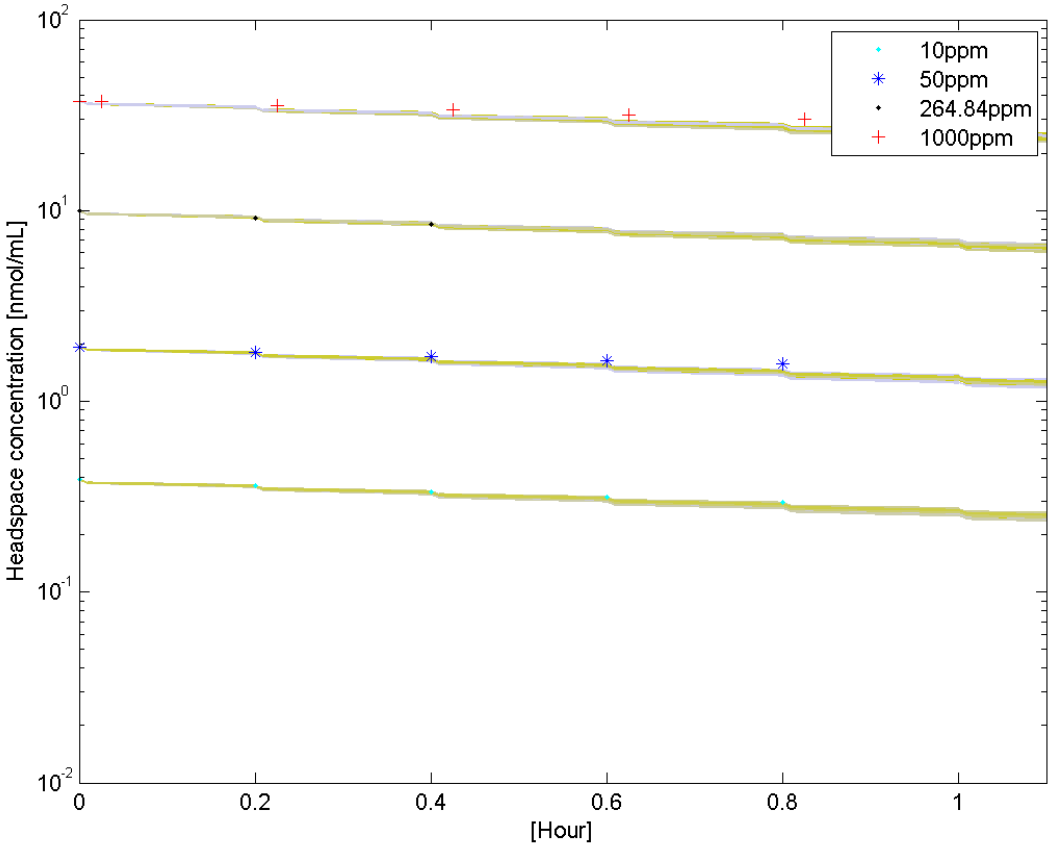
Note: Chloroprene headspace concentrations (symbols) and model simulations (50 lines) based on posterior distribution for parameter values as reported in Table 5. Simulations for each starting concentration represent 50 sets of model parameters randomly drawn from the posterior distributions.

Figure 36
Distribution of chloroprene oxidative metabolism time course in human (pooled mixed gender) liver microsomes



Note: Chloroprene headspace concentrations (symbols) and model simulations (50 lines) based on posterior distribution for parameter values as reported in Table 5. Simulations for each starting concentration represent 50 sets of model parameters randomly drawn from the posterior distributions.

Figure 37
Distribution of chloroprene oxidative metabolism time course in human (pooled mixed gender) lung microsomes



Note: Chloroprene headspace concentrations (symbols) and model simulations (50 lines) based on posterior distribution for parameter values as reported in Table 5. Simulations for each starting concentration represent 50 sets of model parameters randomly drawn from the posterior distributions.

APPENDICES

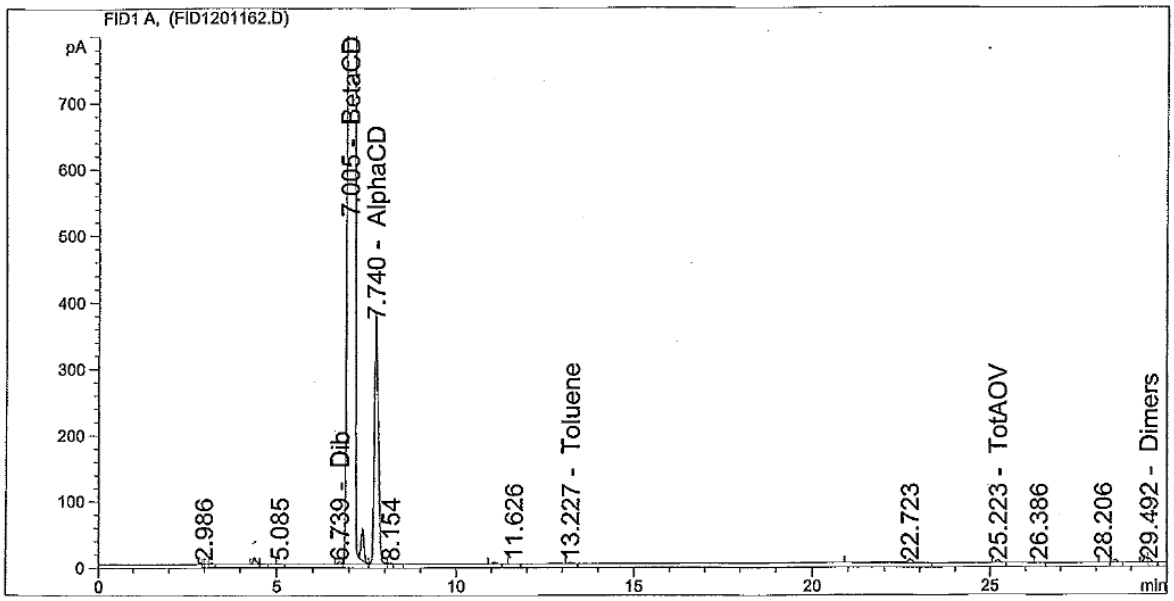
Appendix A
Purity Analysis of β-Chloroprene Provided by the Sponsor's Supplier

Data File C:\CHEM32\2\DATA\FID1[illegible]
Sample Name: PW0902050006

Acq. Operator : BELLTA2
Acq. Instrument : Instrument 2 Location : Vial 101
Injection Date : 05-Feb-09, 10:23:45 Inj : 1
Inj Volume : 1 µl

Method : C:\CHEM32\2\METHODS\1223_FT.M
Last changed : 2/22/2008 7:17:17 AM by FISKSD
Method Info : Adopted from Diamines 1223 Method:
CRUDE CD TO STORAGE - CA,CB
CRUDE CD FROM COND - CC,CD
CD REACTOR EFFLUENT - CQ
CRUDE CD 1373 TK - LA
RECYCLE CD - #1TK-LB,#2TK-LC
CD REFINER MAKE - LG
REFINED CD TO SMU - LH

Sample Info : LH



Normalized Percent Report

Sorted By : Signal
Calib. Data Modified : 2/7/2008 2:55:13 PM
Multiplier : 1.0000
Dilution : 1.0000
Sample Amount : 1.00000 [Wt %] (not used in calc.)
Do not use Multiplier & Dilution Factor with ISTDs

Instrument 2 2/5/2009 10:59:47 AM BELLTA2 Page 1 of 2

Data File C:\CHEM32\2\DATA\FID1201162.D
Sample Name: PW0902050006

Signal 1: FID1 A,

RetTime [min]	Type	Area [pA*s]	Amt/Area	Norm %	Grp	Name
3.070	VV	2.59367	2.26491e-5	0.000023	1	MVA+BT
6.739	BV	20.08306	7.40682e-7	5.91327e-6	1	Dib
7.005	VB S+	3.70246e5	6.73627e-4	99.146237	2	BetaCD
7.364	BV T	278.51874	4.37484e-6	0.000484		1ChlBut2
7.740	VV T	2807.87915	7.12311e-4	0.795086	2	AlphaCD
10.796		-	-	-	3	2CPA
11.070	BB	19.48002	2.23001e-4	0.001727	3	3CPA
12.119		-	-	-		Meso
12.546		-	-	-	3	1CPA
13.227	BB	10.39957	1.21217e-4	0.000501		Toluene
13.698		-	-	-	4	DCbutanes
13.943		-	-	-		34DCB
14.449		-	-	-		Cellosolve
15.760		-	-	-		4VCH
16.701		-	-	-		Cis14DCB
18.000		-	-	-		Trans14DCB
25.223	BB	33.66175	7.50607e-5	0.001004		TotAOV
28.547	BB	31.02670	5.52082e-4	0.006809	5	Dimers
29.389	BV +	50.80355	1.05035e-3	0.021213		CDimers
29.492	VB	37.12034	1.82046e-3	0.026863	5	Dimers

Totals : 100.000000

Group summary :

Group ID	Use	Area [pA*s]	Norm %	Group Name
1		22.67672	2.92657e-5	TotLB
2		3.73054e5	99.941322	TotCD
3		19.48002	1.72688e-3	CPAS
4		0.00000	0.000000	DCBanes
5		68.14704	3.36725e-2	TotDimers

3 Warnings or Errors :

Warning : Calibration warnings (see calibration table listing)
Warning : Time reference compound(s) not found
Warning : Elution order of calibrated compounds may have changed

*** End of Report ***

Instrument 2 2/5/2009 10:59:47 AM BELLTA2 Page 2 of 2

Appendix B
Human Kidney Microsome Data Sheet

XENOTECH
Uncommon Science | Uncommon Service

DATA SHEET

H0610.R / Lot No. 0810236

Human Kidney Microsomes
Mixed Gender, Pool of 8
0.5 mL at 10 mg protein / mL

Specific content and activities [a]	Content / Rate
NADPH-cytochrome *c* reductase (nmol/mg protein/min)	34.5 ± 0.3
Lauric Acid 12-hydroxylation (pmol/mg protein/min)	820 ± 146
Glucuronidation of 4-Methylumbelliferone (nmol/mg protein/min)	125 ± 9

[a] Characterization is performed when the first lot of a product from a given subcellular fraction (*e.g., S9*) is prepared. Subsequent lots are subject to a verification test only. Values for enzyme activities are mean ± standard deviation of three or more determinations.

Donor Information for Human Kidney Microsomes, Lot No. 0810236

Sample	Gender	Age (Yrs)	Race	Cause of Death
10	Female	48	Caucasian	Cerebrovascular accident
11	Female	64	Caucasian	Cerebrovascular accident
12	Male	64	Caucasian	Cerebrovascular accident
13	Female	57	Caucasian	Cerebrovascular accident
14	Male	63	African American	Cerebrovascular accident
15	Female	60	Caucasian	Anoxia
16	Male	62	Caucasian	Cerebrovascular accident
17	Male	69	Caucasian	Cerebrovascular accident

Serology information

- Seven donors tested positive and one donor tested negative for cytomegalovirus
- These donors tested negative for RPR, HIV, HTLV, HbAg, and HCV*

* Rapid Plasma Reagin, Antibody to Human Immunodeficiency Virus, Antibody to Human T Cell Lymphotropic Virus, Hepatitis B Antigen, Antibody to Hepatitis C Virus, respectively.

Store at -80 °C

For in vitro *use only*

CAUTION: *These kidney samples are from donors who tested negative for HIV and hepatitis. However, we recommend that these samples be considered as potential biohazards and that universal precaution be taken when working with human derived products.*

DATA SHEET PREPARED 12.JAN.09

16825 West 116th St. | Lenexa KS 66219
913.GET.P450 | fax 913.227.7100 | xenotechllc.com

Appendix C
Gas Chromatography Gerstel Sampler Calibration Routine

Method: 17520 cd std 200µl injections

Created 4/17/2008 8:02:16 AM
Last changed 4/25/2008 7:45:58 AM

Method Description:

200 µl inj. CD

Heat syringe after injections to clean.

Flush through from 1.0 L CD bag
prepared 25-Apr-08

0 to 60 min.

GC run time = 82 minutes.

Method GC Runtime = 10 min.

Syringe: 1.0ml-HS

01 Home

02 Wait Temperature

Heated Object	Agitator
Temperature (°C)	37

03 Wait Temperature

Heated Object	1.0ml-HS
Temperature (°C)	37

04 Transport Vial

Source Tray	Tray1
Source Index	1
Target Tray	Agitator
Target Index	1

05 Home

06 Start Agitator

Speed (rpm)	400
On Time (s)	20
Off Time (s)	5

07 Wait Agitator

Agitation Time (s)	25

08 Aspirate

Source	Agitator
Index	1
Volume (µl)	200
Air Volume (µl)	0
Filling Speed (µl/s)	100
Filling Strokes	3

09 Needle Bending Prevention

10 Inject

Inject to	HP s/sl
Injection Speed (µl/s)	300
Pre Injection Delay (ms)	100
Post Injection Delay (ms)	200

11 Home

12 Transport Vial

Source Tray	Agitator
Source Index	1
Target Tray	Tray1
Target Index	1

13 Home

14 Gerstel HS Flush

Flush Time (s)	85

15 GC Runtime

GC Runtime (s)	240

Appendix D
Gas Chromatography Gerstel Sampler Microsomal Incubation Routine

Method: 17520 TC exp 200µls 6s 0-60min heat syr vials

Created 4/17/2008 8:04:44 AM
Last changed 4/25/2008 8:27:12 AM

Method Description:

200 µl inj. CD

Heat syringe after injections to clean.

Flush through from 1.0 L CD bag
prepared 25-Apr-08

0 to 60 min.

Method GC Runtime = 10 min.

Syringe: 1.0ml-HS

01 Wait Temperature

Heated Object	Agitator
Temperature (°C)	37.5

02 Wait Temperature

Heated Object	SYR
Temperature (°C)	39

03 Transport Vial

Source Tray	Tray1
Source Index	1
Target Tray	Agitator
Target Index	1

04 Home

05 Start Agitator

Speed (rpm)	500
On Time (s)	30
Off Time (s)	0

06 Wait

Wait Time (s)	2

07 Gerstel HS Flush

Flush Time (s)	290

08 Home

09 Move to Object

Object	Agitator
Index	1

10 Aspirate

Source	Agitator
Index	1
Volume (µl)	200.0
Air Volume (µl)	0
Filling Speed (µl/s)	100
Filling Strokes	3

11 Needle Bending Prevention

12 Inject

Inject to	HP s/sl
Injection Speed (µl/s)	300
Pre Injection Delay (ms)	100

Post Injection Delay (ms)	200
13 Set Temperature	
Heater	1.0ml-HS
Temperature (°C)	48.5
14 Start Agitator	
Speed (rpm)	500
On Time (s)	30
Off Time (s)	0
15 Wait	
Wait Time (s)	2
16 Home	
17 Set Temperature	
Heater	1.0ml-HS
Temperature (°C)	48.5
18 Gerstel HS Flush	
Flush Time (s)	300
19 Set Temperature	
Heater	1.0ml-HS
Temperature (°C)	39.5
20 Gerstel HS Flush	
Flush Time (s)	367
21 Wait	
Wait Time (s)	2
22 Cleanup	
23 Wait	
Wait Time (s)	2
24 Wait Signal	
Sync Signal	Start
25 Home	
26 Move to Object	
Object	Agitator
Index	1
27 Aspirate	
Source	Agitator
Index	1
Volume (µl)	200
Air Volume (µl)	0
Filling Speed (µl/s)	100
Filling Strokes	3
28 Needle Bending Prevention	
29 Inject	
Inject to	HP s/sl
Injection Speed (µl/s)	300
Pre Injection Delay (ms)	100
Post Injection Delay (ms)	200
30 Set Temperature	
Heater	1.0ml-HS
Temperature (°C)	48.5
31 Start Agitator	

Speed (rpm)	500
On Time (s)	30
Off Time (s)	0

32 Home

33 Gerstel HS Flush

Flush Time (s)	300

34 Set Temperature

Heater	1.0ml-HS
Temperature (°C)	39

35 Gerstel HS Flush

Flush Time (s)	320

36 Wait

Wait Time (s)	21

37 Wait

Wait Time (s)	33

38 Start Timer

39 Home

40 Cleanup

41 Home

42 Move to Object

Object	Agitator
Index	1

43 Aspirate

Source	Agitator
Index	1
Volume (µl)	200
Air Volume (µl)	0
Filling Speed (µl/s)	100
Filling Strokes	3

44 Needle Bending Prevention

45 Inject

Inject to	HP s/sl
Injection Speed (µl/s)	300
Pre Injection Delay (ms)	100
Post Injection Delay (ms)	200

46 Set Temperature

Heater	1.0ml-HS
Temperature (°C)	48.5

47 Start Agitator

Speed (rpm)	500
On Time (s)	30
Off Time (s)	0

48 Home

49 Gerstel HS Flush

Flush Time (s)	300

50 Set Temperature

Heater	1.0ml-HS
Temperature (°C)	39

51 Gerstel HS Flush
Flush Time (s) 320

52 Wait
Wait Time (s) 21

53 Wait
Wait Time (s) 33

54 Cleanup

55 Home

56 Move to Object
Object Agitator
Index 1

57 Aspirate
Source Agitator
Index 1
Volume (µl) 200
Air Volume (µl) 0
Filling Speed (µl/s) 100
Filling Strokes 3

58 Needle Bending Prevention

59 Inject
Inject to HP s/sl
Injection Speed (µl/s) 300
Pre Injection Delay (ms) 100
Post Injection Delay (ms) 200

60 Set Temperature
Heater 1.0ml-HS
Temperature (°C) 48.5

61 Start Agitator
Speed (rpm) 500
On Time (s) 30
Off Time (s) 0

62 Start Agitator
Speed (rpm) 500
On Time (s) 30
Off Time (s) 0

63 Home

64 Gerstel HS Flush
Flush Time (s) 300

65 Set Temperature
Heater 1.0ml-HS
Temperature (°C) 39

66 Gerstel HS Flush
Flush Time (s) 320

67 Wait
Wait Time (s) 21

68 Wait
Wait Time (s) 33

69 Cleanup

70 Home

71 Move to Object

Object	Agitator
Index	1

72 Aspirate

Source	Agitator
Index	1
Volume (µl)	200
Air Volume (µl)	0
Filling Speed (µl/s)	100
Filling Strokes	3

73 Needle Bending Prevention

74 Inject

Inject to	HP s/sl
Injection Speed (µl/s)	300
Pre Injection Delay (ms)	100
Post Injection Delay (ms)	200

75 Set Temperature

Heater	1.0ml-HS
Temperature (°C)	48.5

76 Start Agitator

Speed (rpm)	500
On Time (s)	30
Off Time (s)	0

77 Home

78 Gerstel HS Flush

Flush Time (s)	300

79 Set Temperature

Heater	1.0ml-HS
Temperature (°C)	39

80 Gerstel HS Flush

Flush Time (s)	321

81 Wait

Wait Time (s)	21

82 Wait

Wait Time (s)	33

83 Cleanup

84 Home

85 Move to Object

Object	Agitator
Index	1

86 Aspirate

Source	Agitator
Index	1
Volume (µl)	200
Air Volume (µl)	0
Filling Speed (µl/s)	100
Filling Strokes	3

87 Needle Bending Prevention

88 Inject

Inject to	HP s/sl

Injection Speed (µl/s)	300
Pre Injection Delay (ms)	100
Post Injection Delay (ms)	200

89 Set Temperature

Heater	1.0ml-HS
Temperature (°C)	48.5

90 Start Agitator

Speed (rpm)	500
On Time (s)	30
Off Time (s)	0

91 Home

92 Gerstel HS Flush

Flush Time (s)	300

93 Set Temperature

Heater	1.0ml-HS
Temperature (°C)	39

94 Gerstel HS Flush

Flush Time (s)	420

95 Wait

Wait Time (s)	21

96 Wait

Wait Time (s)	34

97 Cleanup

98 Transport Vial

Source Tray	Agitator
Source Index	1
Target Tray	Tray1
Target Index	1

99 Set Temperature

Heater	1.0ml-HS
Temperature (°C)	39

100 Home

Appendix E
Female Rodent Liver and Lung Microsomal Incubation Data Collected at DuPont Haskell Global Centers

Representative Incubation Conditions (for Female B6C3F1 Mouse Liver, 150 ppm)

Female B6 mouse liver microsomes prepared 10/29/07

GC/ECD Me CD_ECD.M
PAL Method 17520_TC_exp_200µl_6s_0-60min_heat_syr_vials

Protein	855.2	µl phosphate buffer	Vial volume	11.648	mL
1.0 mg/ml	29	µl microsome preparation	Liquid volume:	1.000	mL
	0.8	µl G-6-P dehydrogenase (2U)	Headspace volume	10.648	mL
	10	µl glucose-6-phosphate	Protein vol.	0.029	mL
	50	µl $MgCL_2$	Stock prot. Conc.	34.6481	mg/mL
	5	µl EDTA	Amount of protein	1.005	mg
	50	µl NADP+ (25 mg to 3.1 mL Phosphate buffer) - day of use	Protein conc. (mg/ml)	1.005	mg/mL
total	1000				

CD slope
pre-exper. calib.
22-Apr-08
0.000375290094
R2 = 0.999931099448

200 µl Injections

sample #	protein mg/ml	ppm CD	Injection Time			C:\HPCHEM\1\DATA\041908\ Data File # (CDxxxxxx.D)	rt	min	CD area	Headspace Conc (nmol/mL)
19	1.0	150	20:48:46			CD000056	2.088	0	14561.5	5.464787
	w/ NADP+		21:00:43	0:11:57	0:11:57	CD000057	2.087	12	6640.31104	2.492043
			21:12:43	0:23:57	0:12:00	CD000058	2.088	24	2882.80713	1.081889
			21:24:42	0:35:56	0:11:59	CD000059	2.089	36	1300.07703	0.487906
			21:36:44	0:47:58	0:12:02	CD000060	2.089	48	610.01678	0.228933
			21:48:46	1:00:00	0:12:02	CD000061	2.087	60	349.44098	0.131142

sample #	protein mg/ml	ppm CD	Injection Time			Data File # (CDxxxxxx.D)	rt	min	CD area	Headspace Conc (nmol/mL)
21	1.0	150	23:30:47			CD000068	2.088	0	14431.6	5.416037
	w/o NADP+		23:42:46	0:11:59	0:11:59	CD000069	2.089	12	13765.9	5.166206
	added 50 µl PB		23:54:47	0:24:00	0:12:01	CD000070	2.087	24	13247.1	4.971505
			0:06:48	0:36:01	0:12:01	CD000071	2.088	36	12585.3	4.723138
			0:18:49	0:48:02	0:12:01	CD000072	2.087	48	12131.8	4.552944
			0:30:49	1:00:03	0:12:00	CD000073	2.088	60	11639.8	4.368302

Results for Female B6C3F1 Mouse Liver

Incubation status	Minutes of incubation	Chloroprene headspace concentration (nmol/mL)[a] by run date and starting gas bag concentration (ppm) 17-Apr-2008 10 ppm	17-Apr-2008 50 ppm	19-Apr-2008 150 ppm	19-Apr-2008 270 ppm	19-Apr-2008 540 ppm	19-Apr-2008 540 ppm (repeat)	Average 540 ppm
with $NADP^+$	0	0.422	1.867	5.465	9.863	21.170	21.586	21.378
	12	0.052	0.411	2.492	6.070	16.473	17.105	16.789
	24	0.013	0.081	1.082	3.834	13.321	14.222	13.771
	36		0.018	0.488	2.491	9.210	12.039	10.624
	48		0.007	0.229	1.715	9.516	10.464	9.990
	60			0.131	1.185	8.484	9.320	8.902
	% of Initial	3.0	0.4	2.4	12.0	40.1	43.2	41.6

Incubation status	Minutes of incubation	17-Apr-2008 10 ppm	19-Apr-2008 150 ppm	19-Apr-2008 540 ppm
without $NADP^+$	0	0.431	5.416	20.925
	12	0.410	5.166	20.338
	24	0.397	4.972	19.337
	36	0.374	4.723	18.365
	48	0.367	4.553	17.488
	60	0.344	4.368	16.756
	% of Initial	79.8	80.7	80.1

a Microsomal protein concentration = 1 mg/mL

Results for Female B6C3F1 Mouse Lung

Incubation status	Minutes of incubation	Chloroprene headspace concentration (nmol/mL)[a] by run date and starting gas bag concentration (ppm)						
		20-Apr-2008 1 ppm [b]	23-Apr-2008 1 ppm[b] (repeat)	20-Apr-2008 10 ppm	20-Apr-2008 50 ppm	19-Apr-2008 150 ppm	19-Apr-2008 270 ppm	17-Apr-2008 540 ppm
with $NADP^+$	0	0.0396	0.0483	0.434	1.742	5.970	12.522	20.506
	12	0.0318	0.0400	0.346	1.407	5.100	11.105	18.693
	24	0.0272	0.0321	0.297	1.240	4.744	10.556	18.014
	36	0.0251	0.0283	0.272	1.121	4.469	10.119	17.482
	48	0.0229	0.0256	0.250	1.044	4.223	9.644	16.859
	60	0.0214	0.0246	0.229	0.983	4.006	9.284	16.466
	% of Initial	54.0	50.9	52.8	56.4	67.1	74.1	80.3
without $NADP^+$			23-Apr-2008 1 ppm	20-Apr-2008 10 ppm		19-Apr-2008 150 ppm		17-Apr-2008 540 ppm
	0		0.0484	0.433		5.804		20.594
	12		0.0476	0.415		5.478		19.664
	24		0.0461	0.402		5.259		19.003
	36		0.0446	0.388		5.070		18.432
	48		0.0429	0.371		4.775		17.516
	60		0.0407	0.358		4.583		16.778
	% of Initial		84.2	82.7		79.0		81.5

a Microsomal protein concentration = 1 mg/mL
b Combined 1 ppm incubation mean values were 0.044, 0.036, 0.030, 0.027, 0.024, 0.023 nmol/mL at the respective sequential sampling times.

Results for Female F344 Rat Liver

Incubation status	Minutes of incubation	Chloroprene headspace concentration (nmol/mL)[a] by run date and starting gas bag concentration (ppm)				
		22-Apr-2008 1 ppm	22-Apr-2008 10 ppm	22-Apr-2008 50 ppm	21-Apr-2008 150 ppm	21-Apr-2008 270 ppm
with $NADP^+$	0	0.0516	0.465	1.935	6.243	11.007
	12	0.0152	0.141	0.844	4.460	9.091
	24	0.0060	0.048	0.360	3.274	7.661
	36	0.0032	0.022	0.188	2.479	6.621
	48	0.0023	0.011	0.103	1.958	5.831
	60		0.007	0.066	1.607	5.202
	% of Initial	4.5	1.5	3.4	25.7	47.3
without $NADP^+$		23-Apr-2008 1 ppm	22-Apr-2008 10 ppm	22-Apr-2008 50 ppm		21-Apr-2008 270 ppm
	0	0.0479	0.475	1.906		11.061
	12	0.0458	0.449	1.845		10.705
	24	0.0443	0.435	1.747		10.418
	36	0.0427	0.419	1.668		10.158
	48	0.0413	0.404	1.610		9.973
	60	0.0400	0.384	1.533		9.670
	% of Initial	83.5	81.0	80.4		87.4

a Microsomal protein concentration = 1 mg/mL

Result for Female F344 Rat Lung

Incubation status	Minutes of incubation	Chloroprene headspace concentration (nmol/mL)[a] by run date and starting gas bag concentration (ppm)					
		25-Apr-2008 1 ppm	25-Apr-2008 10 ppm	24-Apr-2008 50 ppm	24-Apr-2008 50 ppm b	23-Apr-2008 150 ppm	23-Apr-2008 270 ppm
with $NADP^+$	0	0.0487	0.404	2.051	2.107	5.107	11.438
	12	0.0466	0.378	1.914	1.986	4.611	10.930
	24	0.0449	0.362	1.829	1.910	4.452	10.256
	36	0.0433	0.346	1.755	1.811	4.156	9.786
	48	0.0414	0.335	1.682	1.774	4.131	9.440
	60	0.0398	0.320	1.641	1.655	3.774	8.880
	% of Initial	81.6	79.2	80.0	78.5 b	73.9	77.6
without $NADP^+$		25-Apr-2008 1 ppm	25-Apr-2008 10 ppm	24-Apr-2008 50 ppm	24-Apr-2008 50 ppm [b]		23-Apr-2008 270 ppm
	0	0.0489	0.400	2.061	2.103		11.113
	12	0.0473	0.380	1.953	1.989		10.619
	24	0.0453	0.365	1.899	1.902		10.170
	36	0.0437	0.356	1.820	1.847		9.722
	48	0.0416	0.347	1.732	1.763		9.446
	60	0.0401	0.324	1.666	1.685		9.100
	% of Initial	81.9	81.0	80.8	80.1[b]		81.9

a Microsomal protein concentration = 1 mg/mL except as noted
b Microsomal protein concentration increased from 1 to 3 mg/mL for one set of 50 ppm incubations

Appendix F
Rodent and Human Kidney Microsomal Incubation Data Collected at DuPont Haskell Global Centers

Representative Incubation Conditions (for Male B6C3F1 Mouse Kidney, 150 ppm)

Male B6 mouse kidney microsomes prepared 2/6/09 GC/ECD Method : CD_ECDC.M
(used 6 male microsome vials) PAL Method : 17520_TC_exp_200µl_6s_0-60min_heat_syr_vials

Protein	597	µl phosphate buffer	Vial volume:	11.634	mL
2.0 mg/ml	288	µl microsome preparation	Liquid volume:	1.000	mL
	0.5	µl G-6-P dehydrogenase (2U)	Headspace volume	10.634	mL
	10	µl glucose-6-phosphate	Protein vol.	0.288	mL
	50	µl $MgCL_2$	Stock prot. Conc.	6.945	mg/mL
	5	µl EDTA	Amount of protein	2.000	mg
	50	µl NADP+ (25 mg to 3.1 mL Phosphate buffer) - day of use	Protein conc	2.000	mg/mL
total	1000				

CD slope
pre-exper. calib.
31-Mar-09
0.000439721381
R2 = 0.999104398662

200 µl Injections

sample #	protein mg/ml	ppm CD	Injection Time			C:\HPCHEM\1\DATA\033109\ Data File # (CDxxxxxx.D)	rt	min	CD area	Headspace Conc (nmol/mL)
4	2.0	150	14:11:08			CD000031	2.071	0	12146.6	5.341120
	w/ NADP+		14:23:08	0:12:00	0:12:00	CD000032	2.071	12	10784.1	4.741999
			14:35:10	0:24:02	0:12:02	CD000033	2.071	24	9556.77930	4.202320
			14:47:10	0:36:02	0:12:00	CD000034	2.071	36	8762.62012	3.853111
			14:59:09	0:48:01	0:11:59	CD000035	2.071	48	7971.67041	3.505314
			15:11:11	1:00:03	0:12:02	CD000036	2.072	60	7566.08594	3.326970

sample #	protein mg/ml	ppm CD	Injection Time			Data File # (CDxxxxxx.D)	rt	min	CD area	Headspace Conc (nmol/mL)
5	2.0	150	15:31:14			CD000037	2.070	0	12344.7	5.42823
	w/ Phosphate Buffer		15:43:13	0:11:59	0:11:59	CD000038	2.070	12	11865.1	5.21734
			15:55:11	0:23:57	0:11:58	CD000039	2.069	24	11526.3	5.06836
			16:07:12	0:35:58	0:12:01	CD000040	2.072	36	11339.1	4.98604
			16:19:13	0:47:59	0:12:01	CD000041	2.072	48	10936.0	4.80879
			16:31:17	1:00:03	0:12:04	CD000042	2.071	60	10235.1	4.50059

Results for Male B6C3F1 Mouse Kidney Microsomal Protein Concentration Range Finding

Incubation status	Minutes of incubation	Chloroprene headspace concentration (nmol/mL)[a] by run date and kidney microsomal protein concentration			
		25-Mar-2009 0.5 mg/mL	25-Mar-2009 1.5 mg/mL	25-Mar-2009 2.5 mg/mL	25-Mar-2009 3.0 mg/mL
with $NADP^+$	0	6.413	6.349	6.406	6.567
	12	5.867	5.635	5.654	5.326
	24	5.556	5.312	4.845	4.507
	36	5.381	4.947	4.110	4.000
	48	5.026	4.514	3.659	3.625
	60	4.923	4.081	3.314	3.329
	% of Initial	76.8	64.3	51.7	50.7
without $NADP^+$			25-Mar-2009 1.5 mg/mL		
	0		6.485		
	12		6.059		
	24		5.855		
	36		5.586		
	48		5.283		
	60		5.001		
	% of Initial		77.1		

a Starting concentration for all incubations was 150 ppm

Results for Male B6C3F1 Mouse Kidney

Incubation status	Minutes of incubation	Chloroprene headspace concentration (nmol/mL) by run date and starting gas bag concentration (ppm)								
		1-Apr-2009 2 ppm	10-Apr-2009 2 ppm	Staggered time (min)	1-Apr-2009 2 ppm	1-Apr-2009 10 ppm	1-Apr-2009 50 ppm	31-Mar-2009 150 ppm	31-Mar-2009 270 ppm	31-Mar-2009 540 ppm
			(repeat)							
with $NADP^+$	0	0.0631	0.0877	0	0.0626	0.358	1.764	5.341	10.591	19.674
	12	0.0375	0.0467	6	0.0491	0.207	1.303	4.742	10.001	17.887
	24	0.0250	0.0272	18	0.0296	0.123	1.030	4.202	9.268	16.760
	36	0.0178	0.0182	30	0.0207	0.077	0.822	3.853	8.640	15.391
	48	0.0136	0.0143	42	0.0153	0.055	0.692	3.505	8.100	14.566
	60	0.0108	0.0102	54	0.0124	0.043	0.617	3.327	7.809	13.509
	% of Initial	17.0	11.6		19.9	12.1	35.0	62.3	73.7	68.7

without $NADP^+$		10-Apr-2009 2 ppm	1-Apr-2009 10 ppm	31-Mar-2009 150 ppm	31-Mar-2009 540 ppm
	0	0.0895	0.350	5.428	19.688
	12	0.0863	0.331	5.217	18.933
	24	0.0851	0.325	5.068	18.281
	36	0.0837	0.317	4.986	17.362
	48	0.0806	0.308	4.809	16.752
	60	0.0774	0.296	4.501	15.689
	% of Initial	86.5	84.7	82.9	79.7

Microsomal protein concentration = 2 mg/mL

Results for Female B6C3F1 Mouse Kidney (Microsomal protein, 2 mg/mL)

Incubation status	Minutes of incubation	Chloroprene headspace concentration (nmol/mL) by run date and starting gas bag concentration (ppm)					
		10-Apr-2009 2 ppm	10-Apr-2009 10 ppm	10-Apr-2009 50 ppm	10-Apr-2009 150 ppm	10-Apr-2009 270 ppm	10-Apr-2009 540 ppm
with $NADP^+$	0	0.1003	0.349	1.456	5.102	9.220	19.213
	12	0.0953	0.334	1.398	4.876	8.762	18.370
	24	0.0918	0.318	1.361	4.729	8.439	17.900
	36	0.0885	0.312	1.292	4.568	8.121	17.520
	48	0.0851	0.303	1.258	4.461	7.884	17.147
	60	0.0834	0.287	1.270	4.344	7.643	16.803
	% of Initial	83.2	82.0	87.2	85.2	82.9	87.5
without $NADP^+$		10-Apr 2 ppm	10-Apr 10 ppm		10-Apr 150 ppm		10-Apr 540 ppm
	0	0.1016	0.367		5.175		20.368
	12	0.0976	0.355		4.966		19.492
	24	0.0955	0.348		4.848		18.647
	36	0.0930	0.337		4.737		17.778
	48	0.0899	0.326		4.644		17.106
	60	0.0869	0.316		4.530		16.475
	% of Initial	85.5	85.9		87.5		80.9

Microsomal protein concentration = 2 mg/mL

Results for Female B6C3F1 Mouse Kidney (Microsomal protein, 3 mg/mL)

Incubation status	Minutes of incubation	Chloroprene headspace concentration (nmol/mL) by run date and starting gas bag concentration (ppm)		
		21-Apr-2009 2 ppm	21-Apr-2009 10 ppm	21-Apr-2009 50 ppm
with $NADP^+$	0	0.0900	0.366	1.875
	12	0.0823	0.353	1.868
	24	0.0754	0.344	1.750
	36	0.0702	0.318	1.723
	48	0.0658	0.310	1.632
	60	0.0619	0.298	1.562
	% of Initial	68.8	81.4	83.3
without $NADP^+$		21-Apr-2009 2 ppm	21-Apr-2009 10 ppm	21-Apr-2009 50 ppm
	0	0.0840	0.367	1.847
	12	0.0812	0.352	1.777
	24	0.0792	0.342	1.715
	36	0.0769	0.334	1.642
	48	0.0729	0.326	1.564
	60	0.0704	0.313	1.510
	% of Initial	83.8	85.3	81.8

Microsomal protein concentration = 3 mg/mL

Results for Male F344 Rat Kidney

Incubation status	Minutes of incubation	Chloroprene headspace concentration (nmol/mL) by run date and starting gas bag concentration (ppm)					
		15-Apr-2009 2 ppm	15-Apr-2009 10 ppm	14-Apr-2009 50 ppm	14-Apr-2009 150 ppm	15-Apr-2009 270 ppm	16-Apr-2009 540 ppm
with $NADP^+$	0	0.0961	0.418	1.810	5.405	12.125	22.510
	12	0.0798	0.350	1.631	5.084	11.497	21.626
	24	0.0680	0.298	1.486	4.773	11.072	21.101
	36	0.0586	0.265	1.380	4.515	10.582	20.388
	48	0.0544	0.247	1.284	4.305	10.032	19.658
	60	0.0506	0.228	1.206	4.058	9.587	18.978
	% of Initial	52.6	54.5	66.6	75.1	79.1	84.3
without $NADP^+$		15-Apr-2009 2 ppm	15-Apr-2009 10 ppm		14-Apr-2009 150 ppm	16-Apr-2009 270 ppm	16-Apr-2009 540 ppm
	0	0.0900	0.412		5.356	10.942	23.083
	12	0.0864	0.397		5.115	10.465	22.134
	24	0.0855	0.389		4.999	9.921	21.503
	36	0.0815	0.375		4.896	9.729	20.842
	48	0.0812	0.361		4.751	9.657	20.097
	60	0.0777	0.350		4.517	9.407	19.305
	% of Initial	86.4	84.9		84.3	86.0	83.6

Microsomal protein = 3 mg/mL

Results for Female F344 Rat Kidney

Incubation status	Minutes of incubation	Chloroprene headspace concentration (nmol/mL) by run date and starting gas bag concentration (ppm)					
		18-Apr-2009 2 ppm	17-Apr-2009 10 ppm	17-Apr-2009 50 ppm	17-Apr-2009 150 ppm	16-Apr-2009 270 ppm	16-Apr-2009 540 ppm
with $NADP^+$	0	0.0898	0.436	1.985	5.381	11.003	22.705
	12	0.0784	0.366	1.785	5.102	10.443	21.864
	24	0.0564	0.311	1.636	4.888	10.065	21.230
	36	0.0465	0.266	1.497	4.624	9.656	20.674
	48	0.0438	0.237	1.393	4.387	9.259	19.735
	60	0.0428	0.222	1.297	4.216	8.792	18.879
	% of Initial	47.7	51.1	65.3	78.3	79.9	83.2
without $NADP^+$		18-Apr-2009 2 ppm	17-Apr-2009 10 ppm		17-Apr-2009 150 ppm		16-Apr-2009 540 ppm
	0	0.0886	0.433		5.435		22.816
	12	0.0856	0.418		5.241		22.115
	24	0.0850	0.410		5.139		21.379
	36	0.0829	0.400		4.989		21.165
	48	0.0810	0.388		4.763		20.804
	60	0.0789	0.372		4.591		20.224
	% of Initial	89.1	85.8		84.5		88.6

Microsomal protein = 3 mg/mL

Results for Mixed Gender (Pooled) Human Kidney Microsomes

Incubation status	Minutes of incubation	Chloroprene headspace concentration (nmol/mL) by run date and starting gas bag concentration (ppm)		
		18-Apr 2 ppm	18-Apr 10 ppm	18-Apr 50 ppm
with $NADP^+$	0	0.0930	0.386	2.082
	12	0.0922	0.381	2.054
	24	0.0917	0.375	2.053
	36	0.0893	0.374	1.968
	48	0.0889	0.362	1.963
	60	0.0877	0.354	1.948
	% of Initial	94.3	91.8	93.6
		18-Apr 2 ppm	18-Apr 10 ppm	
without $NADP^+$				
	0	0.0920	0.389	
	12	0.0899	0.378	
	24	0.0883	0.374	
	36	0.0840	0.367	
	48	0.0860	0.362	
	60	0.0847	0.357	
	% of Initial	92.1	91.7	

Microsomal protein = 3 mg/mL

Appendix G
Sample Model Code for MCMC Analysis

```
Sample Model Code for MCMC Analysis

I :  CSL file
II :  MCMC file
III :  CallMCMC file

I :  CSL file is the actual PK model file
program invitro.csl
VARIABLE TIME

INITIAL

CONSTANT VMAX1a=0.          !'MAX RATE OF MET. (uMOL/HR/mg protein)'
CONSTANT VMAX1b=0.          !'MAX RATE OF MET. (uMOL/HR/mg protein)'
CONSTANT KM1a=0.1           !'MICHAELIS CONSTANT (uMOL/L)'
CONSTANT KM1b=0.1           !'MICHAELIS CONSTANT (uMOL/L)'

CONSTANT VK=0.            !'REPRESENT THE V/K COEFFICIENT FOR RAT LUNG (1/hr)'
CONSTANT RLOSS=0.           !'REPRESENT THE background loss rate (1/hr)'
CONSTANT P1=0.69           !'MEDIA/AIR PARTITION for CD'

CONSTANT A10=0.            !'INITIAL AMOUNT IN VIAL (uMOL)'

CONSTANT VVIAL=0.01163   !'VOLUME OF VIAL (L); Vial volume= 11.65 ml'
CONSTANT VMED=0.001        !'VOLUME OF MEDIA (L); Liquid voume'
 VAIR=VVIAL-VMED         !'HEADSPACE'
CONSTANT PROT = 1.0        !'AMOUNT OF PROTEIN (mg)'

CONSTANT TF=0.            !'TIME OF FIRST SAMPLE (hr); kept same'
CONSTANT TI=0.2            !'INTERVAL BETWEEN SAMPLES (hr)kept same'
CONSTANT VINJ=0.0002       !'INJECTION VOLUME (L); based on Matt email'

!'Initial Conditions'
 CA10=A10/(VAIR+P1*VMED)
 CM10=CA10*P1                    !'CONC in SOLUTION'
 CA1=CA10
 CM1=CM10
 A1I=0.



!'TIMING COMMANDS'
CONSTANT TSTOP=1.1        !'LENGTH OF EXPOSURE (HOURS)'
CONSTANT POINTS=100.       !'NO. OF POINTS IN PLOT'
CINTERVAL CINT=0.01
TS=TF
SCHEDULE step .AT. TF

END                       !'END INITIAL'

DYNAMIC

ALGORITHM IALG=2

  DERIVATIVE

  TERMT(TIME.GE.TSTOP)

!  'CD KINETICS (umoles/hr)'
  R1Ma=(VMAX1a*CM1)/(KM1a+CM1)*PROT
  R1Mb=(VMAX1b*CM1)/(KM1b+CM1)*PROT
  RRLUNGVK=VK*CM1
  RRLOSS=RLOSS*CM1
  A1Ma=INTEG(R1Ma,0.)
  A1Mb=INTEG(R1Mb,0.)
  ARLUNGVK=INTEG(RRLUNGVK,0.)
  ARLOSS=INTEG(RRLOSS, 0.) !background loss rate

  CA1=(A10-A1Ma-A1Mb-ARLUNGVK-A1I-ARLOSS)/(VAIR+VMED*P1)
```

```
  CM1=CA1*P1
  A1=CA1*VAIR+CM1*VMED


!  'MASS BALANCE'
  CHECK1 = A10 - (A1+A1Ma+A1Mb+A1I+ ARLUNGVK+ARLOSS)

DISCRETE step
PROCEDURAL
!'Routine for sample loss'
    A1I=A1I+CA1*VINJ
    SCHEDULE step .AT. TS+TI
    TS=TS+TI
END                         !'END PROCEDURAL'
END                         !'END DISCRETE'

END                         !'END DERIVATIVE'
END                         !'END DYNAMIC'
END                         !'END PROGRAM'
```

```
II :  MCMC file :  MCMC setting and function
function tchains = runmcmc(pchains = [])
    % Driver code for MCMC analysis
    global zdata
    global firstT
    global lastT
    global firstD
    global lastD
    global CCC
    global LI
    global sLV
    global sLK
    global Vmax
    global Km
    global sVmax
    global sKm
    global sVK
    global preds
    LI = zeros(1, 1);
    sLV = zeros(1, 1);
    sLK = zeros(1, 1);
    Vmax = zeros(1, 1);
    Km = zeros(1, 1);
    sVmax = zeros(2, 1);
    sKm = zeros(2, 1);

    numParms = 9
    numChains = 1
    numIts = 2000000
    funcNames = ["mcInit", "mcEvalLikelihoods", "mcEvalPriors", "mcSamplePriors",
"mcEvalProposal", "mcSampleProposal"]
    updateMode = 4
    chains = mcmc(numParms, numIts, numChains, updateMode, funcNames, pchains);
    save @format=ascii @file=mcmc_results.dat chains
    tchains = chains([1:50:2000000],:);
end

function mcInit()
    global zdata
    global firstT
    global lastT
    global firstD
    global lastD
    global CCC
    global LI
    global sLV
    global sLK
    global Vmax
    global Km
    global sVmax
    global sKm
    global sVK
    global preds
    global OpMcmcPriorBounds
    OpMcmcPriorBounds = [...
    0.01, 10
    0.01, 10
    0.01, 10
    -10, 5
    -10, 5
    -20, 10
    -20, 10
    -20, 10
    -20, 10
    ];
    global OpMcmcAdaptive
    OpMcmcAdaptive = 1;
    global OpMcmcDelayedRejection
    OpMcmcDelayedRejection = 0;
    global OpMcmcAdaptPeriod
    OpMcmcAdaptPeriod = 30;
```

```
    global OpMcmcAdaptCovarScale
    OpMcmcAdaptCovarScale = 1;
    global OpMcmcLoggingPeriod
    OpMcmcLoggingPeriod = 50;
    global OpMcmcAdaptLowerThresh
    OpMcmcAdaptLowerThresh = 0.25;
    global OpMcmcAdaptUpperThresh
    OpMcmcAdaptUpperThresh = 0.45;
    global OpMcmcAdaptLowerThreshDR
    OpMcmcAdaptLowerThreshDR = 0.45;
    global OpMcmcAdaptUpperThreshDR
    OpMcmcAdaptUpperThreshDR = 0.65;
    global OpMcmcSigmaDecreaseFact
    OpMcmcSigmaDecreaseFact = 0.9;
    global OpMcmcSigmaIncreaseFact
    OpMcmcSigmaIncreaseFact = 1.1;
    global OpMcmcDRSigmaReduceFact
    OpMcmcDRSigmaReduceFact = 0.2;
    global OpMcmcDRSigmaReduceFactAM
    OpMcmcDRSigmaReduceFactAM = 0.1;
    global OpMcmcAdaptLowerThreshAM
    OpMcmcAdaptLowerThreshAM = 0.15;
    global OpMcmcAdaptUpperThreshAM
    OpMcmcAdaptUpperThreshAM = 0.3;
    global OpMcmcCovarScaleDecreaseFact
    OpMcmcCovarScaleDecreaseFact = 20;
    global OpMcmcCovarScaleIncreaseFact
    OpMcmcCovarScaleIncreaseFact = 20;
    global OpDemcSnookerFraction
    OpDemcSnookerFraction = 0.1;
    global OpDemcThinningFactor
    OpDemcThinningFactor = 10;
    global OpDemcB
    OpDemcB = 0.0001;
end

function samp = mcSampleProposal(prevsamp)
    global zdata
    global firstT
    global lastT
    global firstD
    global lastD
    global CCC
    global LI
    global sLV
    global sLK
    global Vmax
    global Km
    global sVmax
    global sKm
    global sVK
    global preds
    samp = [];
    % This function is a stub...
    % Code for a user-defined proposal function can be inserted here.
end

function val = mcEvalProposal(samp, prevsamp)
    global zdata
    global firstT
    global lastT
    global firstD
    global lastD
    global CCC
    global LI
    global sLV
    global sLK
    global Vmax
    global Km
    global sVmax
    global sKm
```

```
    global sVK
    global preds
    val = 0;
    % This function is a stub...
    % Code for a user-defined proposal function can be inserted here.
end

function mcDumpSamples()
    global zdata
    global firstT
    global lastT
    global firstD
    global lastD
    global CCC
    global LI
    global sLV
    global sLK
    global Vmax
    global Km
    global sVmax
    global sKm
    global sVK
    global preds
    LI
    sLV
    sLK
    Vmax
    Km
    sVmax
    sKm
end

function names = mcSampNames()
    names = "LI";
    names = [names, "sLV"];
    names = [names, "sLK"];
    names = [names, "Vmax"];
    names = [names, "Km"];
    names = [names, "sVmax(1)"];
    names = [names, "sVmax(2)"];
    names = [names, "sKm(1)"];
    names = [names, "sKm(2)"];
    names
end

function parms = mcPackSamples()
    global zdata
    global firstT
    global lastT
    global firstD
    global lastD
    global CCC
    global LI
    global sLV
    global sLK
    global Vmax
    global Km
    global sVmax
    global sKm
    global sVK
    global preds
    parms = [];
    parms = [parms LI];
    parms = [parms sLV];
    parms = [parms sLK];
    parms = [parms Vmax];
    parms = [parms Km];
    parms = [parms reshape(sVmax, 1, 2)];
    parms = [parms reshape(sKm, 1, 2)];
end
```

```
function mcUnpackSamples(parms)
    global zdata
    global firstT
    global lastT
    global firstD
    global lastD
    global CCC
    global LI
    global sLV
    global sLK
    global Vmax
    global Km
    global sVmax
    global sKm
    global sVK
    global preds
    idx = 1;
    LI = parms(idx); idx = idx + 1;
    sLV = parms(idx); idx = idx + 1;
    sLK = parms(idx); idx = idx + 1;
    Vmax = parms(idx); idx = idx + 1;
    Km = parms(idx); idx = idx + 1;
    sVmax = reshape(parms(idx:idx+1), 2, 1); idx = idx + 2;
    sKm = reshape(parms(idx:idx+1), 2, 1); idx = idx + 2;
end

function parms = mcSamplePriors()
    global zdata
    global firstT
    global lastT
    global firstD
    global lastD
    global CCC
    global LI
    global sLV
    global sLK
    global Vmax
    global Km
    global sVmax
    global sKm
    global sVK
    global preds
    LI = normrnd(1, 1);
    Vmax = unifrnd(-10, 5);
    Km = unifrnd(-10, 5);
    sLV = lognrnd(-1.2, 1.6);
    sLK = lognrnd(-1.2, 1.6);
    for gg = 1 :  2
        sVmax(gg) = normrnd(Vmax, sLV);
        sKm(gg) = normrnd(Km, sLK);
    end
    parms = mcPackSamples();
end

function val = mcEvalPriors(parms)
    global zdata
    global firstT
    global lastT
    global firstD
    global lastD
    global CCC
    global LI
    global sLV
    global sLK
    global Vmax
    global Km
    global sVmax
    global sKm
    global sVK
    global preds
    mcUnpackSamples(parms);
```

```
    val = 0.0;
    val = val + normlpdf(LI, 1, 1);
    val = val + uniflpdf(Vmax, -10, 5);
    val = val + uniflpdf(Km, -10, 5);
    val = val + lognlpdf(sLV, -1.2, 1.6);
    val = val + lognlpdf(sLK, -1.2, 1.6);
    for gg = 1 :  2
        val = val + normlpdf(sVmax(gg), Vmax, sLV);
        val = val + normlpdf(sKm(gg), Km, sLK);
    end
end

function val = mcEvalLikelihoods(parms)
    global zdata
    global firstT
    global lastT
    global firstD
    global lastD
    global CCC
    global LI
    global sLV
    global sLK
    global Vmax
    global Km
    global sVmax
    global sKm
    global sVK
    global preds
    mcUnpackSamples(parms);
    val = 0.0;
    sVK = 0;
    for gg = 1 :  2
        for i = firstD(gg) :  lastD(gg)
            preds = getpreds(sVmax(gg), sKm(gg), sVK, CCC(i), gg);
            for j = firstT(gg) :  lastT(gg)
                if(~isnan(zdata(j, i)))
                    val = val + normlpdf(zdata(j, i), preds(j), LI);
                end
            end
        end
    end
end
```

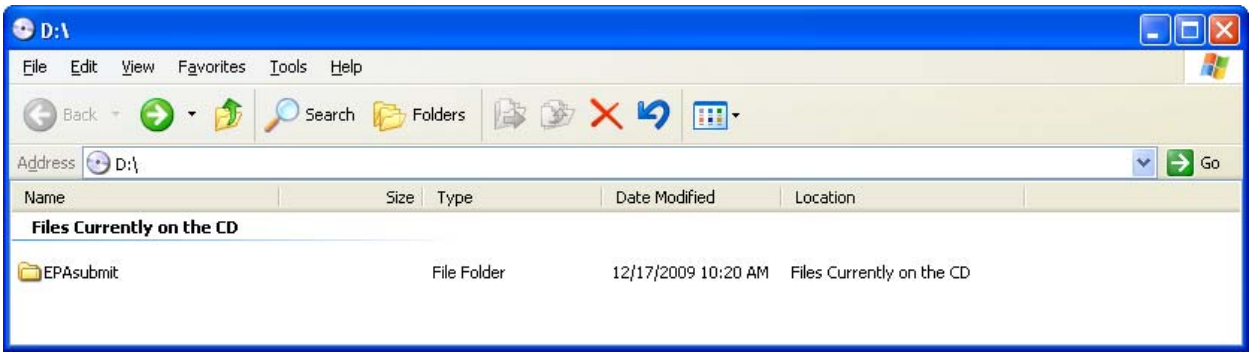
```
III :  CallMCMC file :  link CSL model to MCMC

load @format = model @file =
/home/yyang/work/Chloroprene/ACSL/MCMC/RatBothLiver/chain1/invitro.so

prepare @clear
prepare @all

disp('Both Fisher Rat, Liver Case')
seedrnd(4556)

VVIALF=0.01165; %% Male ==VVIAL=.0119573;
VVIALM=0.0119573;
VMED=.001;
VINJF=0.0002; %% Male ==VIN=0.0003858 !important
VINM=0.0003858 ;
VAIRF=VVIALF-VMED;
VAIRM=VVIALM-VMED;
TSTOP=1.2;
TF=0.;
TI=0.2;
PROT = 1.0;
P1 = 0.69;
WESITG=0;
WEDITG =0;


start @nocallback

global _ca1
global _time
global zdata
global tFindex
global tMindex

global firstT
global lastT
global firstD
global lastD
global CCC
global ControlData

use ('/home/yyang/work/Chloroprene/ACSL/MCMC/Control/ControlData.m')

%CDF Liver Summary
%Time   1 ppm   10 ppm  50 ppm  150 ppm 270 ppm
FratFLiver=[
0.      0.052   0.465   1.935   6.243   11.007  ;
0.2     0.015   0.141   0.844   4.460   9.091   ;
0.4     0.006   0.048   0.360   3.274   7.661   ;
0.6     0.003   0.022   0.188   2.479   6.621   ;
0.8     0.002   0.011   0.103   1.958   5.831   ;
1.      NaN     0.007   0.066   1.607   5.202   ];

%[Time  264 ppm 132 ppm 50 ppm
FratMLiver = [
0       2.0125  4.6755  9.824;
0.025   2.18    4.503   9.454;
0.05    1.634   4.318   8.939;
0.1     1.354   3.918   9.767;
0.15    1.113   3.708   9.603;
0.2     0.893   3.217   7.856;
0.225   0.931   3.007   7.581;
0.25    0.706   2.885   7.02;
0.3     0.545   2.559   7.925;
0.35    0.419   2.478   7.679;
0.4     0.291   2.0245  6.097;
0.425   0.308   1.841   5.974;
0.45    0.237   1.786   5.568;
0.5     0.175   1.547   6.201;
0.55    0.125   1.558   NaN;
```

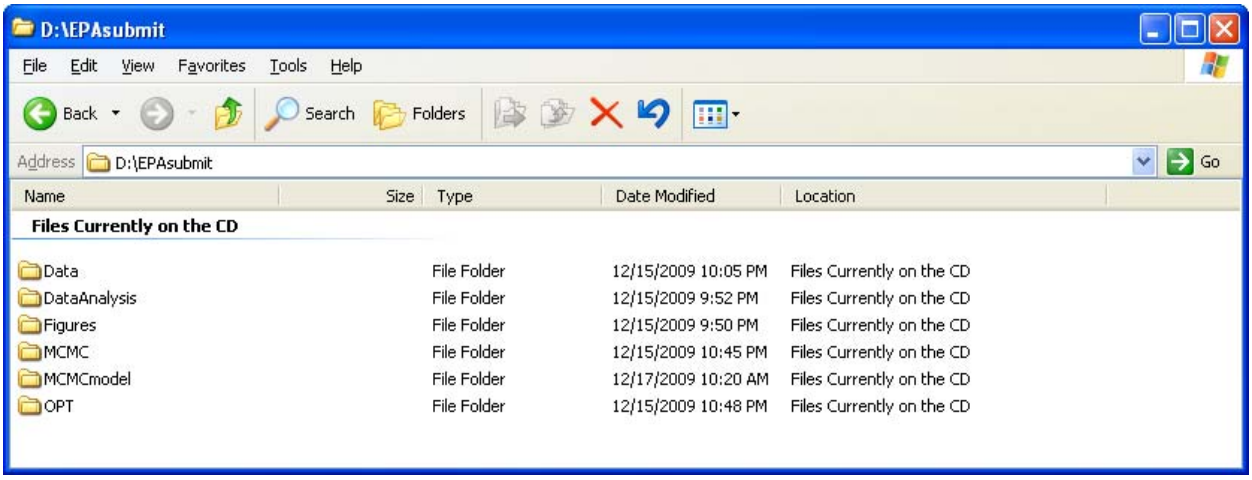
```
0.6    0.077   1.1375  4.637;
0.625  0.082   1.01    4.584;
0.65   0.067   0.995   4.231;
0.7    0.048   0.837   NaN;
0.75   0.034   0.708   NaN;
0.8    0.0195  0.5715  3.482;
0.825  0.02    0.483   3.428;
0.85   0.018   0.489   3.18;
0.9    NaN     0.397   NaN;
0.95   0.009   NaN     NaN];



tempF= size(FratFLiver);
tempM= size(FratMLiver);

ID_Time = 1;
ID_DoseF = [(ID_Time+1):1:tempF(2)];
ID_DoseM = [(ID_Time+1):1:tempM(2)];

dataF = FratFLiver(:, ID_DoseF);
dataM = FratMLiver(:, ID_DoseM);

tempF= size(dataF);
tempM= size(dataM);

tFindex = FratFLiver(:, ID_Time);
tMindex = FratMLiver(:, ID_Time);

% number of time points :max(tempM(1), tempF(1))
% number of dose :  (tempM(2)+tempF(2))

zdata = NaN* ones([max(tempM(1), tempF(1)), (tempM(2)+tempF(2))]); % corresponse to max 25
timepoints and 5 dose each gender
zdata(1:tempF(1), 1:tempF(2)) = dataF ;% first Female, then Male
zdata(1:tempM(1), tempF(2)+1:tempF(2)+tempM(2)) = dataM;

firstT = [1, 1];% time point;% first Female, then Male
lastT  = [tempF(1), tempM(1)];
firstD = [1, tempF(2)+1];% dose groups% first Female, then Male
lastD = [tempF(2), tempF(2)+tempM(2)];

AAF=dataF(1,:)*(VAIRF+P1*VMED);
AAB=dataM(1,:)*(VAIRM+P1*VMED);

CCC = [AAF, AAB];
zdata=log(zdata);


function preds = getpreds(Vmax, Km,VK, A10, Gender)
   global _ca1
   global _time
   global tFindex
   global tMindex
   global ControlData

   % draw back ground loss rate
   tmp = ceil(rand*500);
   lossR = ControlData(tmp);
   setmdl("RLOSS", exp(lossR));

   setmdl("VMAX1A", exp(Vmax));  % reset model parameter as global variables
   setmdl("KM1A", exp(Km));
   setmdl("VK", VK);
   setmdl("A10", A10);

   if Gender==1
      tindex = tFindex;
      setmdl("VVIAL", 0.01165);
      setmdl("VINJ", 0.0002);
   else
```

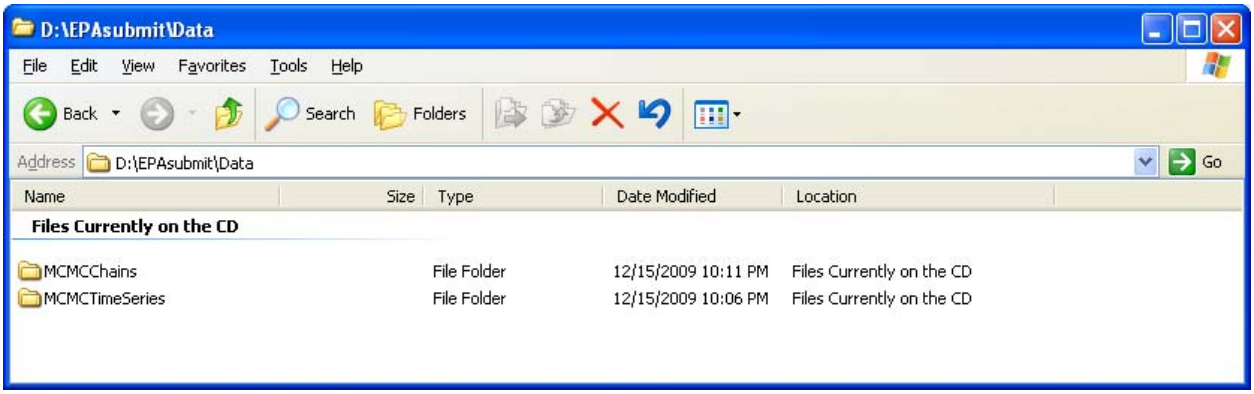
```
      tindex = tMindex;
      setmdl("VVIAL",.0119573);
      setmdl("VINJ", 0.0003858);
   end

  data @clear
  data("SAMPTIMES", ["T"], tindex);

  start @nocallback

  preds = NaN*ones(length(tindex), 1);

for i = 1:length(tindex)
    idx = find(_time == tindex(i));
    if(idx ~= [])
      preds(i) = max(0.0, _ca1(idx));
    end
end

preds = log(preds);

end


use invitromc11.m
chains = runmcmc();
```

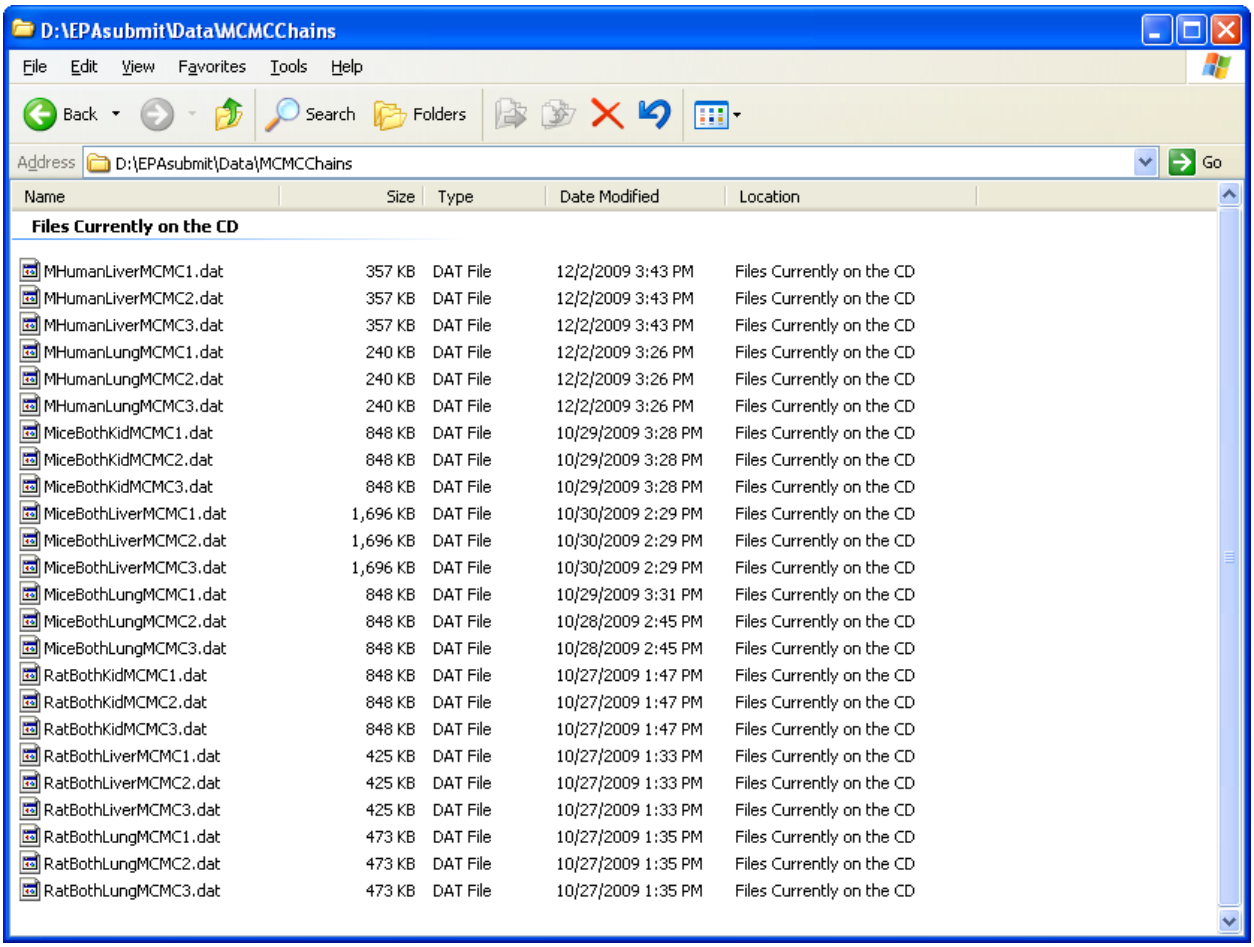
Appendix H
Screen Capture Documentation of Model Code and Full MCMC Chain Results

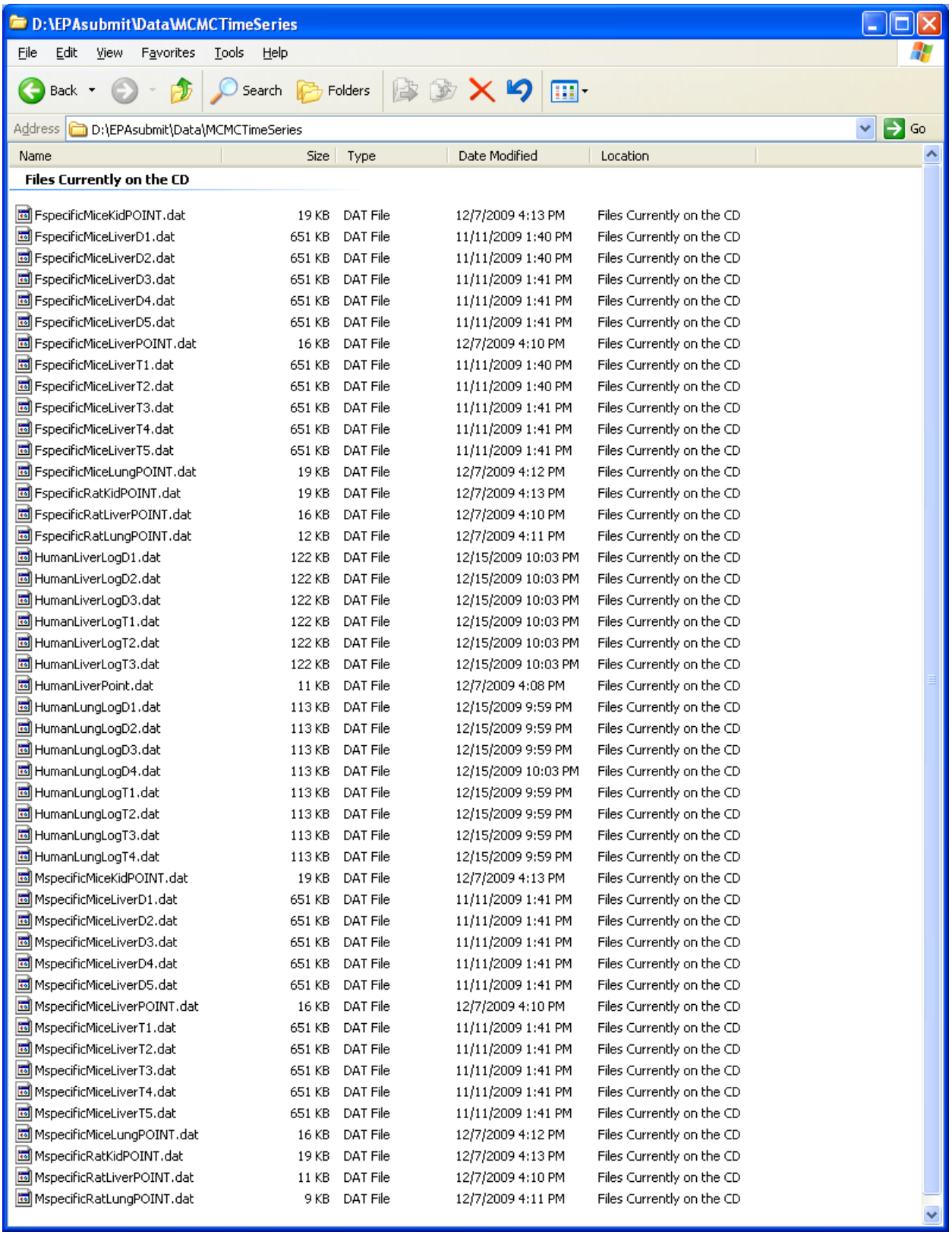
Screen Captures of Modeling Files (Total File Size is 1.4 Gb)








D:\EPAsubmit\Data\MCMCChains
File Edit View Favorites Tools Help
Back Search Folders
Address D:\EPAsubmit\Data\MCMCChains
Go
Name Size Type Date Modified Location
Files Currently on the CD
MHumanLiverMCMC1.dat 357 KB DAT File 12/2/2009 3:43 PM Files Currently on the CD
MHumanLiverMCMC2.dat 357 KB DAT File 12/2/2009 3:43 PM Files Currently on the CD
MHumanLiverMCMC3.dat 357 KB DAT File 12/2/2009 3:43 PM Files Currently on the CD
MHumanLungMCMC1.dat 240 KB DAT File 12/2/2009 3:26 PM Files Currently on the CD
MHumanLungMCMC2.dat 240 KB DAT File 12/2/2009 3:26 PM Files Currently on the CD
MHumanLungMCMC3.dat 240 KB DAT File 12/2/2009 3:26 PM Files Currently on the CD
MiceBothKidMCMC1.dat 848 KB DAT File 10/29/2009 3:28 PM Files Currently on the CD
MiceBothKidMCMC2.dat 848 KB DAT File 10/29/2009 3:28 PM Files Currently on the CD
MiceBothKidMCMC3.dat 848 KB DAT File 10/29/2009 3:28 PM Files Currently on the CD
MiceBothLiverMCMC1.dat 1,696 KB DAT File 10/30/2009 2:29 PM Files Currently on the CD
MiceBothLiverMCMC2.dat 1,696 KB DAT File 10/30/2009 2:29 PM Files Currently on the CD
MiceBothLiverMCMC3.dat 1,696 KB DAT File 10/30/2009 2:29 PM Files Currently on the CD
MiceBothLungMCMC1.dat 848 KB DAT File 10/29/2009 3:31 PM Files Currently on the CD
MiceBothLungMCMC2.dat 848 KB DAT File 10/28/2009 2:45 PM Files Currently on the CD
MiceBothLungMCMC3.dat 848 KB DAT File 10/28/2009 2:45 PM Files Currently on the CD
RatBothKidMCMC1.dat 848 KB DAT File 10/27/2009 1:47 PM Files Currently on the CD
RatBothKidMCMC2.dat 848 KB DAT File 10/27/2009 1:47 PM Files Currently on the CD
RatBothKidMCMC3.dat 848 KB DAT File 10/27/2009 1:47 PM Files Currently on the CD
RatBothLiverMCMC1.dat 425 KB DAT File 10/27/2009 1:33 PM Files Currently on the CD
RatBothLiverMCMC2.dat 425 KB DAT File 10/27/2009 1:33 PM Files Currently on the CD
RatBothLiverMCMC3.dat 425 KB DAT File 10/27/2009 1:33 PM Files Currently on the CD
RatBothLungMCMC1.dat 473 KB DAT File 10/27/2009 1:35 PM Files Currently on the CD
RatBothLungMCMC2.dat 473 KB DAT File 10/27/2009 1:35 PM Files Currently on the CD
RatBothLungMCMC3.dat 473 KB DAT File 10/27/2009 1:35 PM Files Currently on the CD



D:\EPAsubmit\Data\MCMCTimeSeries

File Edit View Favorites Tools Help

Back Search Folders

Address D:\EPAsubmit\Data\MCMCTimeSeries Go

Name	Size	Type	Date Modified	Location
Files Currently on the CD				
FspecificMiceKidPOINT.dat	19 KB	DAT File	12/7/2009 4:13 PM	Files Currently on the CD
FspecificMiceLiverD1.dat	651 KB	DAT File	11/11/2009 1:40 PM	Files Currently on the CD
FspecificMiceLiverD2.dat	651 KB	DAT File	11/11/2009 1:40 PM	Files Currently on the CD
FspecificMiceLiverD3.dat	651 KB	DAT File	11/11/2009 1:41 PM	Files Currently on the CD
FspecificMiceLiverD4.dat	651 KB	DAT File	11/11/2009 1:41 PM	Files Currently on the CD
FspecificMiceLiverD5.dat	651 KB	DAT File	11/11/2009 1:41 PM	Files Currently on the CD
FspecificMiceLiverPOINT.dat	16 KB	DAT File	12/7/2009 4:10 PM	Files Currently on the CD
FspecificMiceLiverT1.dat	651 KB	DAT File	11/11/2009 1:40 PM	Files Currently on the CD
FspecificMiceLiverT2.dat	651 KB	DAT File	11/11/2009 1:40 PM	Files Currently on the CD
FspecificMiceLiverT3.dat	651 KB	DAT File	11/11/2009 1:41 PM	Files Currently on the CD
FspecificMiceLiverT4.dat	651 KB	DAT File	11/11/2009 1:41 PM	Files Currently on the CD
FspecificMiceLiverT5.dat	651 KB	DAT File	11/11/2009 1:41 PM	Files Currently on the CD
FspecificMiceLungPOINT.dat	19 KB	DAT File	12/7/2009 4:12 PM	Files Currently on the CD
FspecificRatKidPOINT.dat	19 KB	DAT File	12/7/2009 4:13 PM	Files Currently on the CD
FspecificRatLiverPOINT.dat	16 KB	DAT File	12/7/2009 4:10 PM	Files Currently on the CD
FspecificRatLungPOINT.dat	12 KB	DAT File	12/7/2009 4:11 PM	Files Currently on the CD
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HumanLiverLogD3.dat	122 KB	DAT File	12/15/2009 10:03 PM	Files Currently on the CD
HumanLiverLogT1.dat	122 KB	DAT File	12/15/2009 10:03 PM	Files Currently on the CD
HumanLiverLogT2.dat	122 KB	DAT File	12/15/2009 10:03 PM	Files Currently on the CD
HumanLiverLogT3.dat	122 KB	DAT File	12/15/2009 10:03 PM	Files Currently on the CD
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HumanLungLogD2.dat	113 KB	DAT File	12/15/2009 9:59 PM	Files Currently on the CD
HumanLungLogD3.dat	113 KB	DAT File	12/15/2009 9:59 PM	Files Currently on the CD
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HumanLungLogT3.dat	113 KB	DAT File	12/15/2009 9:59 PM	Files Currently on the CD
HumanLungLogT4.dat	113 KB	DAT File	12/15/2009 9:59 PM	Files Currently on the CD
MspecificMiceKidPOINT.dat	19 KB	DAT File	12/7/2009 4:13 PM	Files Currently on the CD
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MspecificMiceLiverD2.dat	651 KB	DAT File	11/11/2009 1:41 PM	Files Currently on the CD
MspecificMiceLiverD3.dat	651 KB	DAT File	11/11/2009 1:41 PM	Files Currently on the CD
MspecificMiceLiverD4.dat	651 KB	DAT File	11/11/2009 1:41 PM	Files Currently on the CD
MspecificMiceLiverD5.dat	651 KB	DAT File	11/11/2009 1:41 PM	Files Currently on the CD
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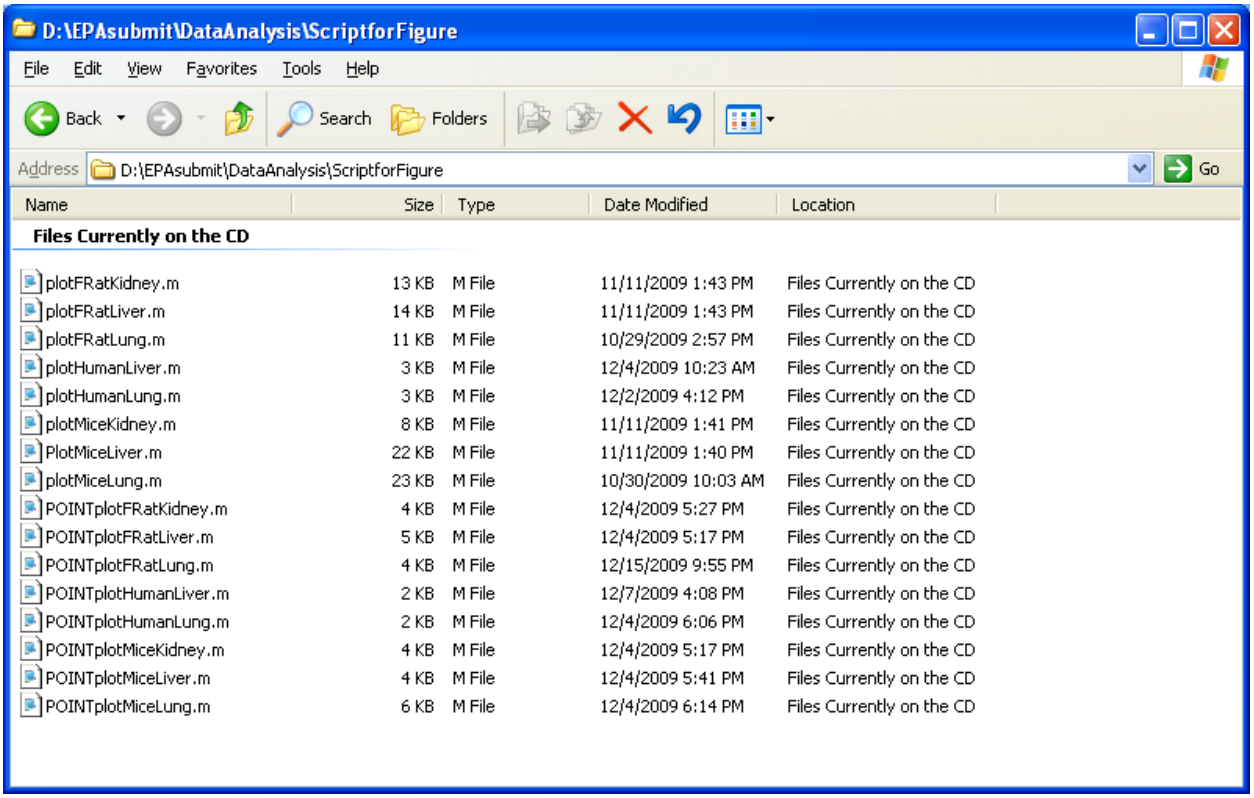
MspecificRatLungPOINT.dat	9 KB	DAT File	12/7/2009 4:11 PM	Files Currently on the CD



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plotMiceLung.m	23 KB	M File	10/30/2009 10:03 AM	Files Currently on the CD
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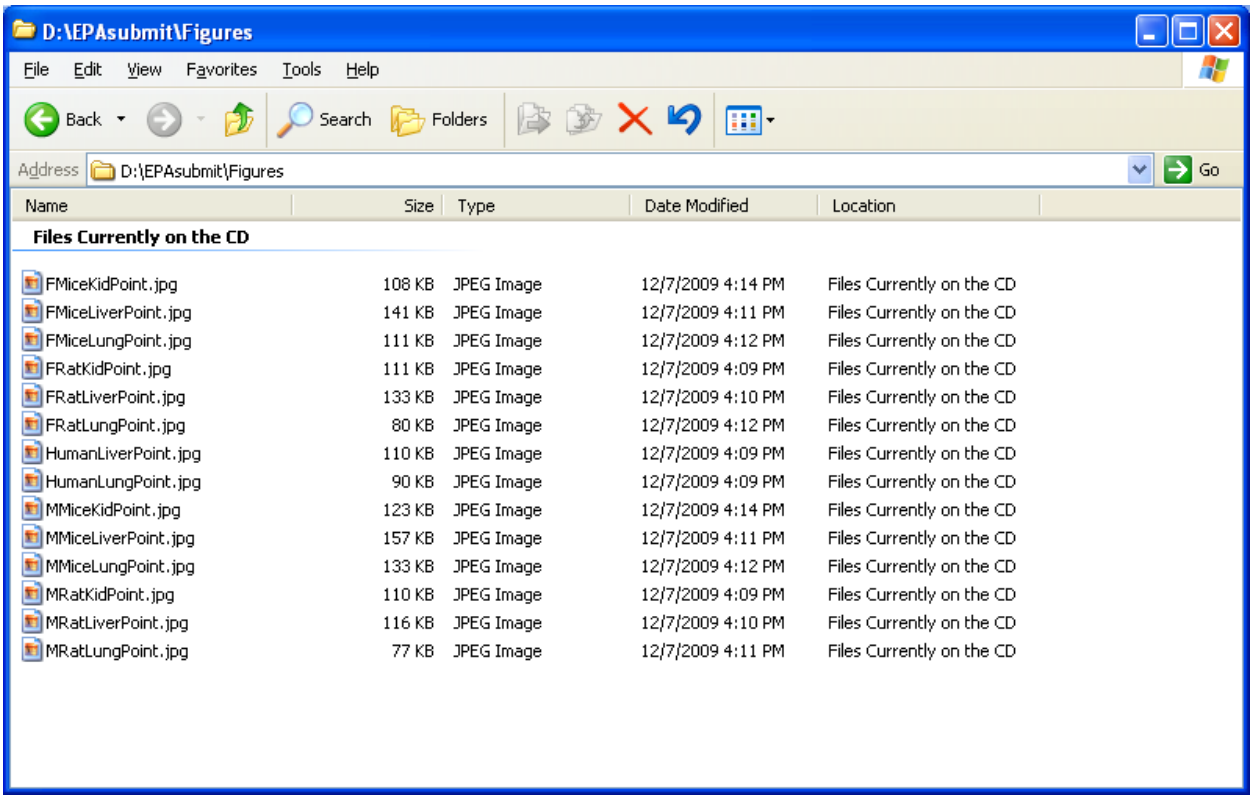

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FMiceLungPoint.jpg	111 KB	JPEG Image	12/7/2009 4:12 PM	Files Currently on the CD
FRatKidPoint.jpg	111 KB	JPEG Image	12/7/2009 4:09 PM	Files Currently on the CD
FRatLiverPoint.jpg	133 KB	JPEG Image	12/7/2009 4:10 PM	Files Currently on the CD
FRatLungPoint.jpg	80 KB	JPEG Image	12/7/2009 4:12 PM	Files Currently on the CD
HumanLiverPoint.jpg	110 KB	JPEG Image	12/7/2009 4:09 PM	Files Currently on the CD
HumanLungPoint.jpg	90 KB	JPEG Image	12/7/2009 4:09 PM	Files Currently on the CD
MMiceKidPoint.jpg	123 KB	JPEG Image	12/7/2009 4:14 PM	Files Currently on the CD
MMiceLiverPoint.jpg	157 KB	JPEG Image	12/7/2009 4:11 PM	Files Currently on the CD
MMiceLungPoint.jpg	133 KB	JPEG Image	12/7/2009 4:12 PM	Files Currently on the CD
MRatKidPoint.jpg	110 KB	JPEG Image	12/7/2009 4:09 PM	Files Currently on the CD
MRatLiverPoint.jpg	116 KB	JPEG Image	12/7/2009 4:10 PM	Files Currently on the CD
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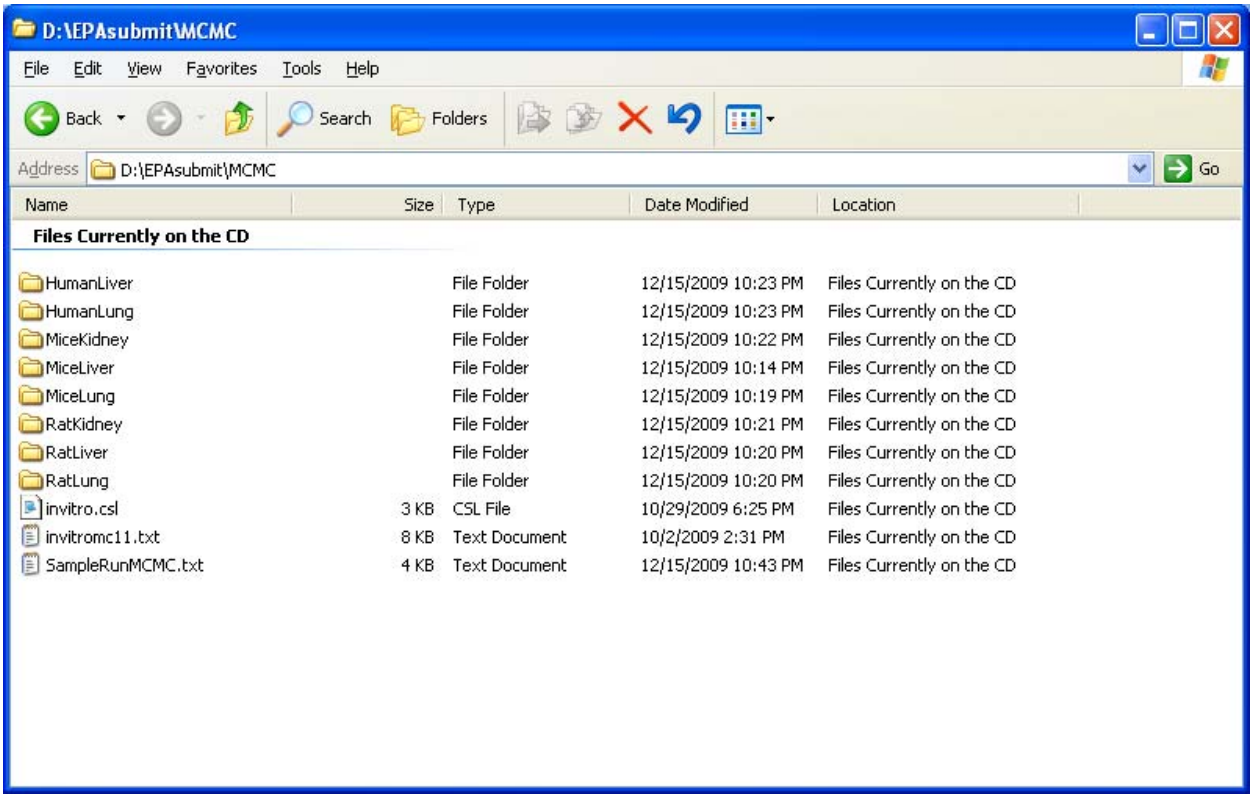
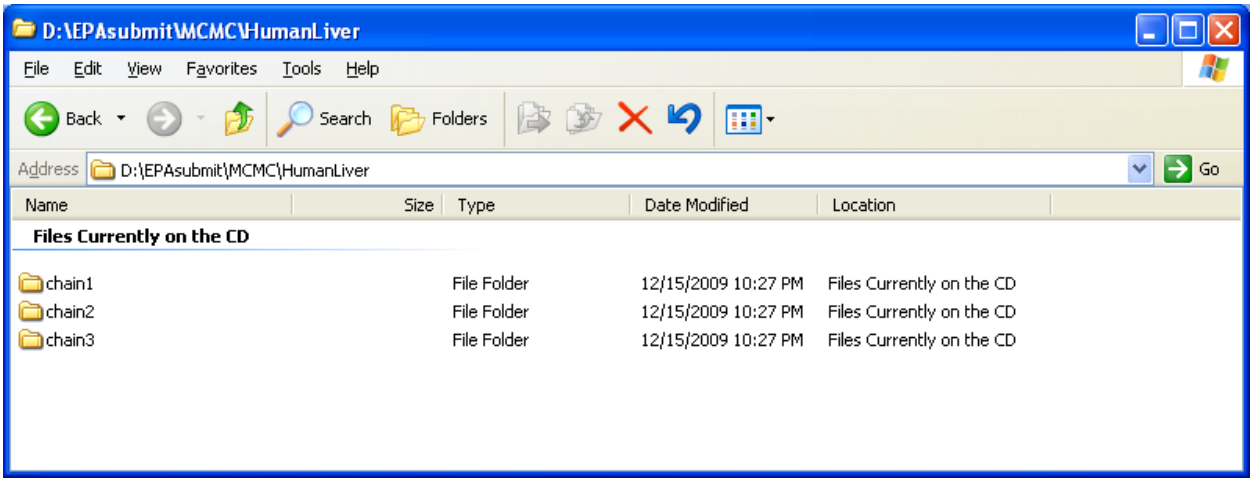
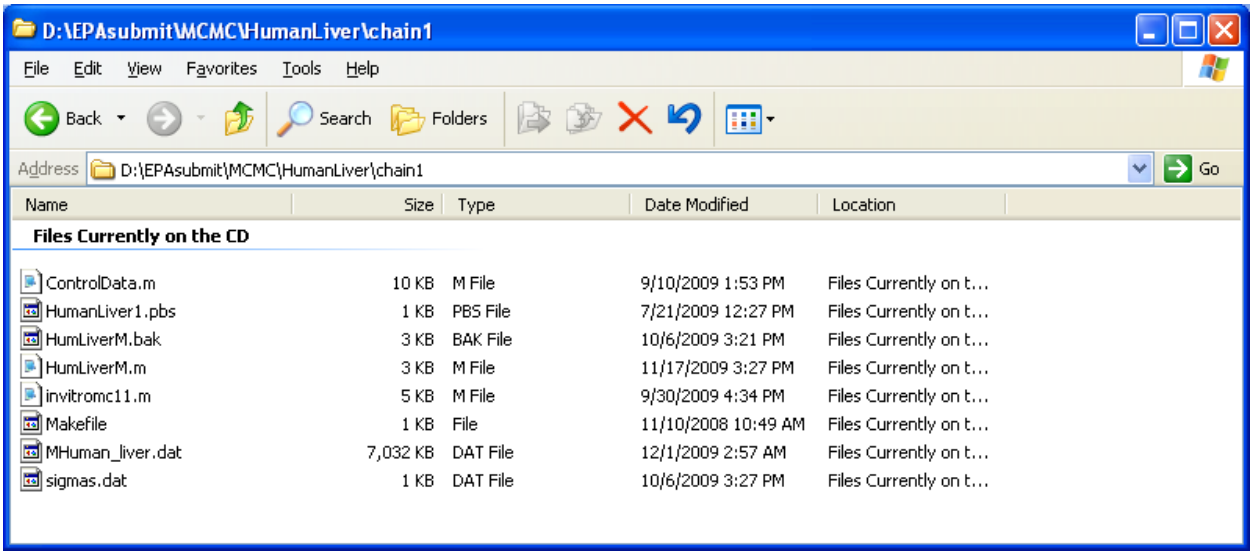
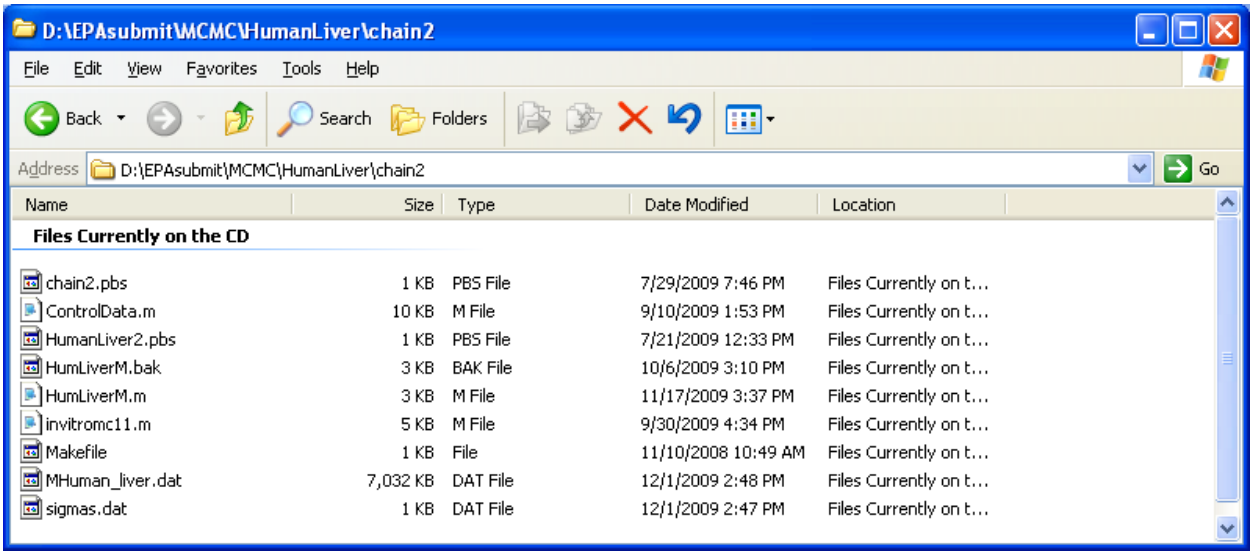


Representative chain folder for human from folder above (additional specie folders not presented here as individual screen captures)




D:\EPAsubmit\MCMC\HumanLiver\chain1
File Edit View Favorites Tools Help
Back Search Folders
Address D:\EPAsubmit\MCMC\HumanLiver\chain1
Go
Name Size Type Date Modified Location
Files Currently on the CD
ControlData.m 10 KB M File 9/10/2009 1:53 PM Files Currently on t...
HumanLiver1.pbs 1 KB PBS File 7/21/2009 12:27 PM Files Currently on t...
HumLiverM.bak 3 KB BAK File 10/6/2009 3:21 PM Files Currently on t...
HumLiverM.m 3 KB M File 11/17/2009 3:27 PM Files Currently on t...
invitromc11.m 5 KB M File 9/30/2009 4:34 PM Files Currently on t...
Makefile 1 KB File 11/10/2008 10:49 AM Files Currently on t...
MHuman_liver.dat 7,032 KB DAT File 12/1/2009 2:57 AM Files Currently on t...
sigmas.dat 1 KB DAT File 10/6/2009 3:27 PM Files Currently on t...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Back Search Folders
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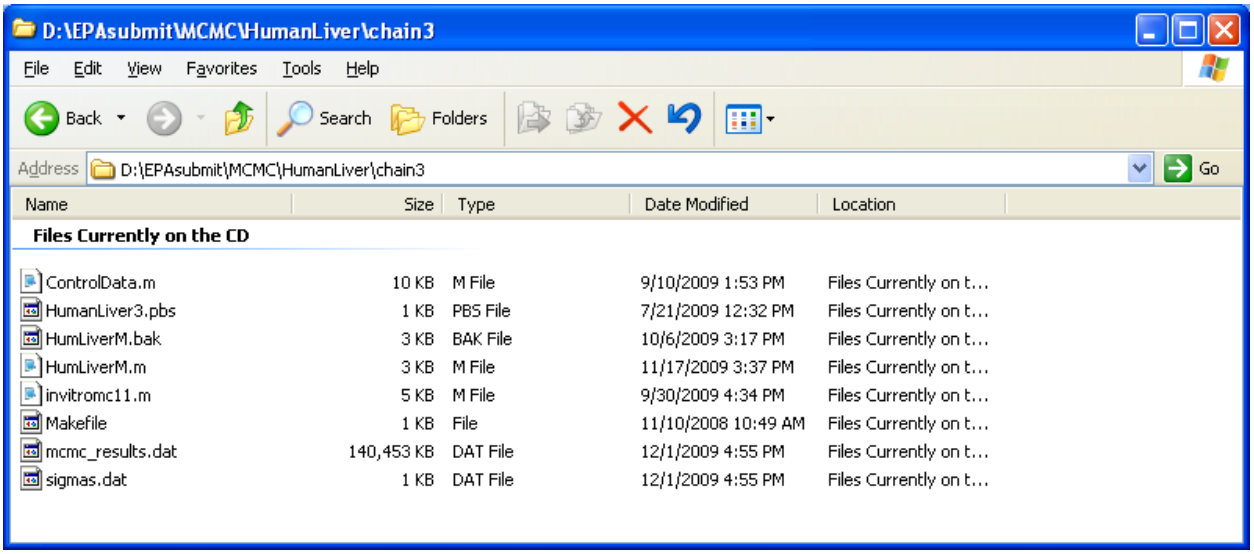
Files Currently on the CD
chain2.pbs 1 KB PBS File 7/29/2009 7:46 PM Files Currently on t...
ControlData.m 10 KB M File 9/10/2009 1:53 PM Files Currently on t...
HumanLiver2.pbs 1 KB PBS File 7/21/2009 12:33 PM Files Currently on t...
HumLiverM.bak 3 KB BAK File 10/6/2009 3:10 PM Files Currently on t...
HumLiverM.m 3 KB M File 11/17/2009 3:37 PM Files Currently on t...
invitromc11.m 5 KB M File 9/30/2009 4:34 PM Files Currently on t...
Makefile 1 KB File 11/10/2008 10:49 AM Files Currently on t...
MHuman_liver.dat 7,032 KB DAT File 12/1/2009 2:48 PM Files Currently on t...
sigmas.dat 1 KB DAT File 12/1/2009 2:47 PM Files Currently on t...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Back Search Folders
Address D:\EPAsubmit\MCMC\HumanLiver\chain3
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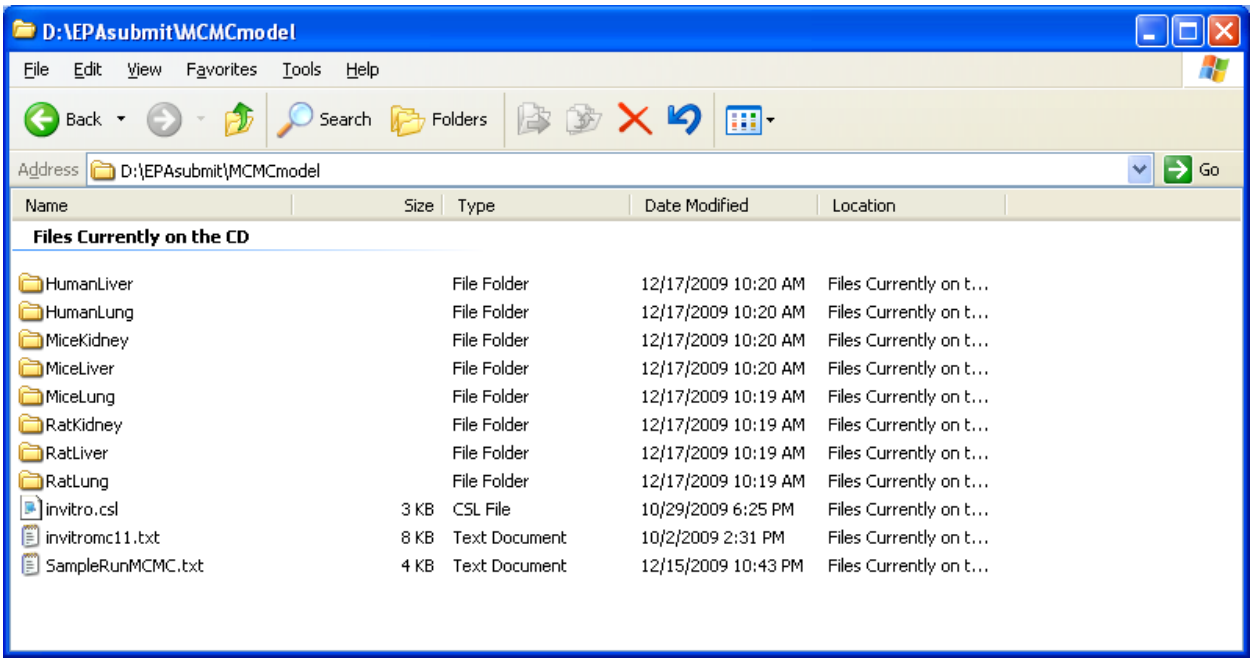
Files Currently on the CD
ControlData.m 10 KB M File 9/10/2009 1:53 PM Files Currently on t...
HumanLiver3.pbs 1 KB PBS File 7/21/2009 12:32 PM Files Currently on t...
HumLiverM.bak 3 KB BAK File 10/6/2009 3:17 PM Files Currently on t...
HumLiverM.m 3 KB M File 11/17/2009 3:37 PM Files Currently on t...
invitromc11.m 5 KB M File 9/30/2009 4:34 PM Files Currently on t...
Makefile 1 KB File 11/10/2008 10:49 AM Files Currently on t...
mcmc_results.dat 140,453 KB DAT File 12/1/2009 4:55 PM Files Currently on t...
sigmas.dat 1 KB DAT File 12/1/2009 4:55 PM Files Currently on t...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File Edit View Favorites Tools Help
Back Search Folders
Address D:\EPAsubmit\MCMCmodel
Go
Name Size Type Date Modified Location
Files Currently on the CD
HumanLiver File Folder 12/17/2009 10:20 AM Files Currently on t...
HumanLung File Folder 12/17/2009 10:20 AM Files Currently on t...
MiceKidney File Folder 12/17/2009 10:20 AM Files Currently on t...
MiceLiver File Folder 12/17/2009 10:20 AM Files Currently on t...
MiceLung File Folder 12/17/2009 10:19 AM Files Currently on t...
RatKidney File Folder 12/17/2009 10:19 AM Files Currently on t...
RatLiver File Folder 12/17/2009 10:19 AM Files Currently on t...
RatLung File Folder 12/17/2009 10:19 AM Files Currently on t...
invitro.csl 3 KB CSL File 10/29/2009 6:25 PM Files Currently on t...
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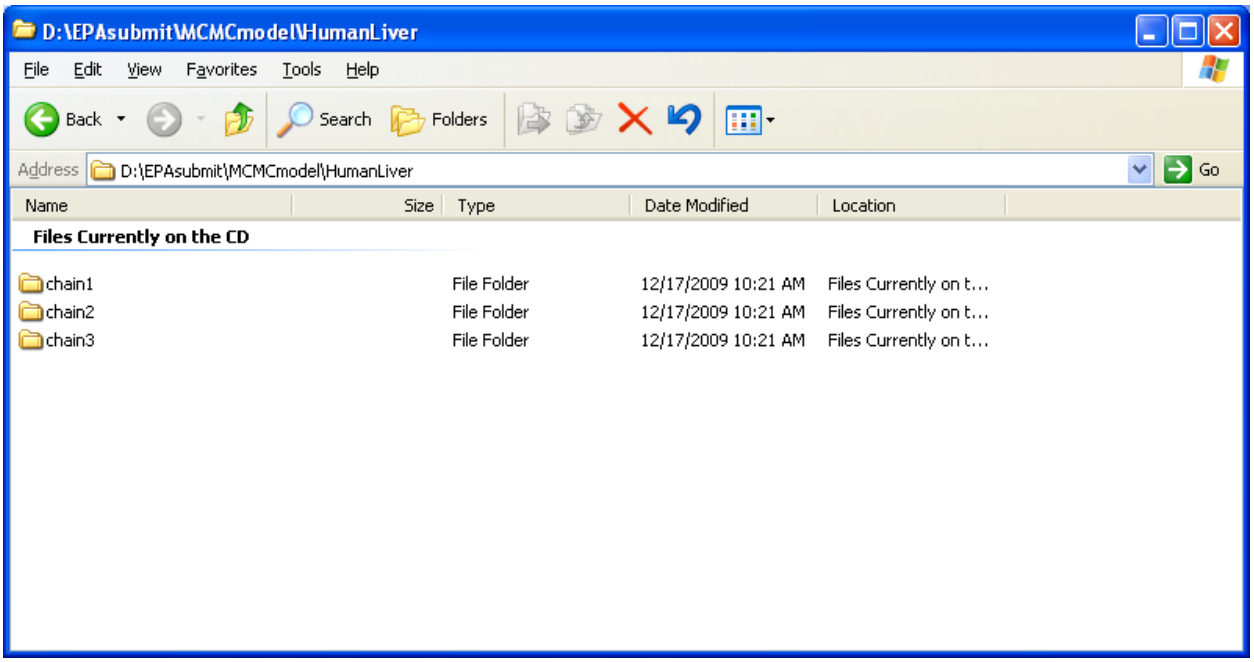
SampleRunMCMC.txt 4 KB Text Document 12/15/2009 10:43 PM Files Currently on t...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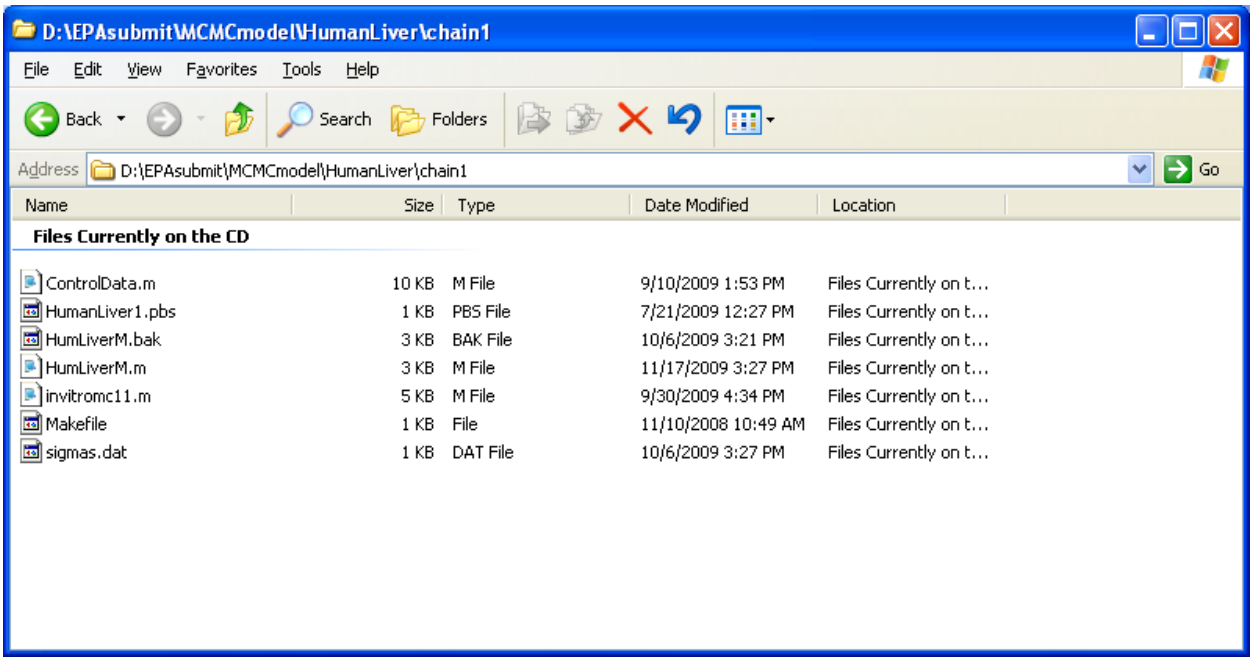
Back Search Folders
Address D:\EPAsubmit\MCMCmodel\HumanLiver
Go
Name Size Type Date Modified Location
Files Currently on the CD
chain1 File Folder 12/17/2009 10:21 AM Files Currently on t...
chain2 File Folder 12/17/2009 10:21 AM Files Currently on t...
chain3 File Folder 12/17/2009 10:21 AM Files Currently on t...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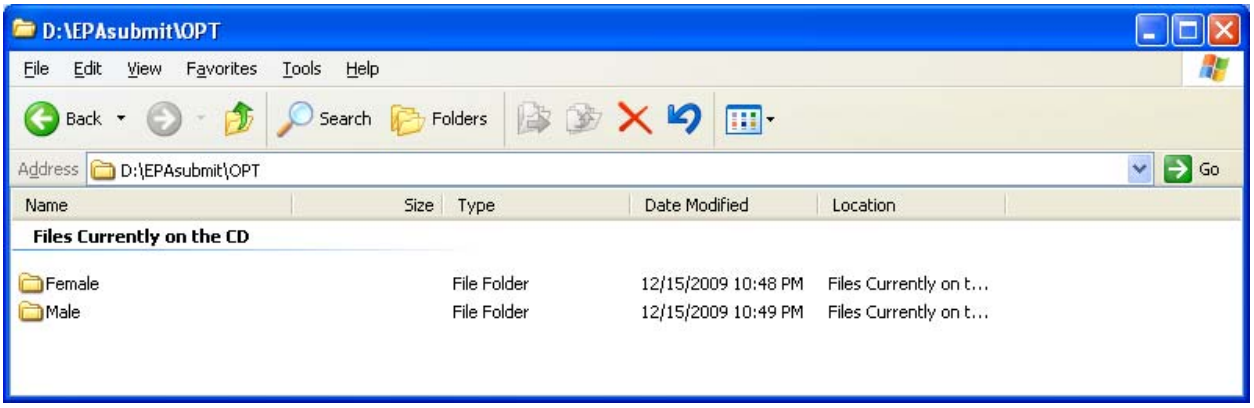
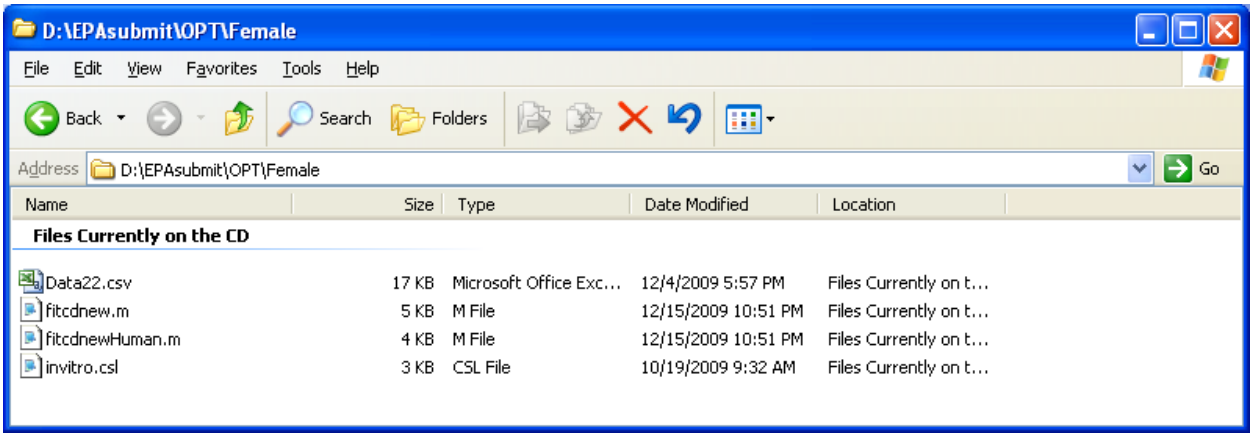
File Edit View Favorites Tools Help
Back Search Folders
Address D:\EPAsubmit\MCMCmodel\HumanLiver\chain1
Go
Name Size Type Date Modified Location
Files Currently on the CD
ControlData.m 10 KB M File 9/10/2009 1:53 PM Files Currently on t...
HumanLiver1.pbs 1 KB PBS File 7/21/2009 12:27 PM Files Currently on t...
HumLiverM.bak 3 KB BAK File 10/6/2009 3:21 PM Files Currently on t...
HumLiverM.m 3 KB M File 11/17/2009 3:27 PM Files Currently on t...
invitromc11.m 5 KB M File 9/30/2009 4:34 PM Files Currently on t...
Makefile 1 KB File 11/10/2008 10:49 AM Files Currently on t...
sigmas.dat 1 KB DAT File 10/6/2009 3:27 PM Files Currently on t...


D:\EPAsubmit\OPT
File Edit View Favorites Tools Help
Back Search Folders
Address D:\EPAsubmit\OPT
Go
Name Size Type Date Modified Location
Files Currently on the CD
Female File Folder 12/15/2009 10:48 PM Files Currently on t...
Male File Folder 12/15/2009 10:49 PM Files Currently on t...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File Edit View Favorites Tools Help
Back Search Folders
Address D:\EPAsubmit\OPT\Female
Go
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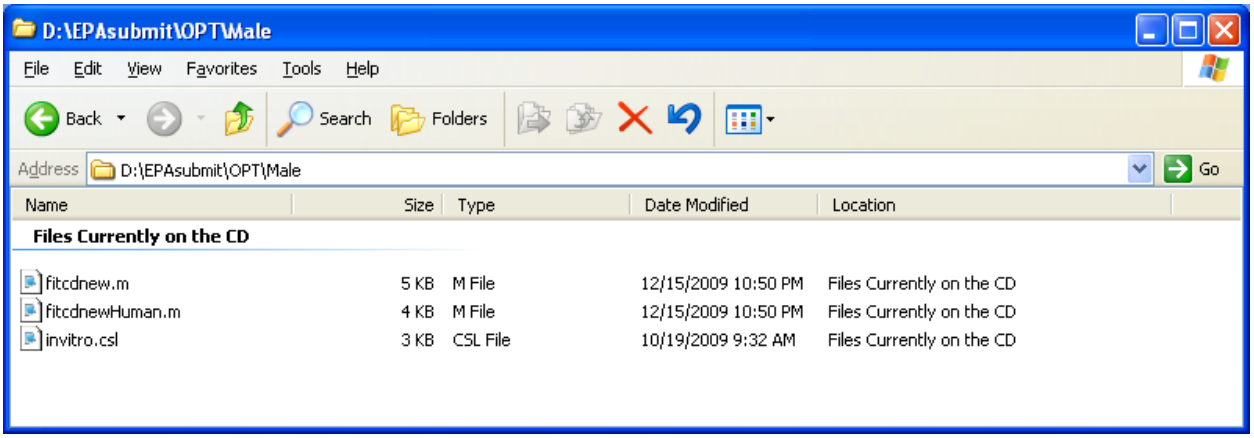
Data22.csv 17 KB Microsoft Office Exc... 12/4/2009 5:57 PM Files Currently on t...
fitcdnew.m 5 KB M File 12/15/2009 10:51 PM Files Currently on t...
fitcdnewHuman.m 4 KB M File 12/15/2009 10:51 PM Files Currently on t...
invitro.csl 3 KB CSL File 10/19/2009 9:32 AM Files Currently on t...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File Edit View Favorites Tools Help
Back Search Folders
Address D:\EPAsubmit\OPT\Male
Go
Name Size Type Date Modified Location
Files Currently on the CD
fitcdnew.m 5 KB M File 12/15/2009 10:50 PM Files Currently on the CD
fitcdnewHuman.m 4 KB M File 12/15/2009 10:50 PM Files Currently on the CD
invitro.csl 3 KB CSL File 10/19/2009 9:32 AM Files Currently on the CD